THE REMEDIATION OF INDUSTRIALLY CONTAMINATED SOIL

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To my husband

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ABSTRACT

The Remediation of Industrially Contaminated Soil

The remediation of two contaminated soils in the Tyne and Wear Metropolitan district was examined. These were a sediment dredged from the river bed at Dunston Coal Staiths on the River Tyne (downstream from Derwenthaugh coke work site) and coke work-contaminated soil from the Derwenthaugh site, Blaydon, Nr. Newcastle-upon-Tyne.

The river Tyne dredgings were of a very fine material (70% silt; 24% clay) with high water retention capacity. Levels of (EDTA available) Zn (490mg/kg), total Cd (7.5mg/kg) and total Pb (510mg/kg) were above the Department of Environment`s (1987) threshold values for soil contaminants. Barley (*Hordeum vulgare* L. *cv* Kym) sown in the dredgings in ten outdoor plots (1m x 0.5m), grew very poorly (yield = 2.4g dry wt./plant, compared with that on an uncontaminated control soil (7.4g dry wt./plant). The barley exhibited all the classic signs of metal phytotoxicity despite the addition of fertiliser and organic waste (straw and spent mushroom compost).

When lime was added to raise the pH of the dredgings in the plots to over pH 7.1, the growth rate and the yield of barley improved significantly (yield = 6.8g dry wt./plant). Levels of available Zn, Cd, Pb and Cu in the limed dredgings were now lower than in the unlimed dredgings. Copper and zinc levels in leaves of barley raised on the limed material were lower than levels in barley grown on unlimed dredgings. There was no significant difference in yield or growth rate between the different plots of dredgings in which organic supplementation parameters were varied. In conclusion, pH was the dominant factor in the remediation of the heavy metal phytotoxicity in the dredged material.

Gas chromatography/mass spectrophotometry analysis showed the principal contaminants of the coke works soil to be organic. The soil was heavily contaminated with coal tars (19.0%) consisting of a complex mixture of aliphatic, polycyclic and aromatic compounds including phenols (160mg/kg). Viable counts of the soil microflora, on selective media, showed the presence of bacteria capable of degrading phenol and several of its alkylated homologues and thiocyanate, which was converted to ammonia and used as a N source.

The coke works soil was treated on a laboratory scale using microbially based clean-up methods. Soil was incubated in glass jars under laboratory conditions. Nutrients (yeast extract) and microbial biomass (a mixed culture, previously isolated and enriched by growth on cresol and thiocyanate, but capable of oxidising a wide range of alkylated phenols), were inoculated into the contaminated soil. The addition of such biomass (10^6 organisms /g soil) led to a marked improvement in the rate of phenolic degradation in the soil (26% loss in 2 weeks, compared with 9% in the untreated control.). Degradation rates decreased after 14 days but a repeated application of biomass (10^6 organisms/g soil) caused further phenolic loss (47% total loss). Cresol (100mg/kg) subsequently added to the bacterially-amended soil disappeared within 7 days, showing that the biomass amendment was still biochemically very active.

These findings demonstrate the importance and the effectiveness of two different treatment methods in the remediation of contaminated soil.

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INTRODUCTION

1.1 Introduction

In the last 250 years, Europe has been transformed from a predominantly agricultural community to an industrial society. Europe, particularly Britain and Germany, rapidly established a heavy manufacturing industry. This was followed by an equally swift decline. Poor emission controls during their active industrial working life and the subsequent decommissioning of a wide range of large and small industries, e.g. gas works, mining and smelting, have left a legacy of site dereliction and widespread soil contamination. A number of factors have, however, recently converged to make the redevelopment of these former industrial sites an attractive proposition.

During post-Second World War years, both Labour and Conservative governments have implemented strong house building policies to accommodate the growing population of distinct family groups with rising living standards. The escalation in the cost of imported foods has necessarily encouraged greater home production. With policies aimed at protecting the green belt and agricultural land, by limiting building development on them, frequently the only land left for major industrial and housing development has been former industrial land.

Such sites are often in prime city centre locations where, because demand for land often exceeds supply, these sites have become, in spite of the greater development costs compared with a `green field` site, more attractive and have received more attention from developers because of escalating land values (Finnecy & Parker, 1986). The redevelopment and hence revitalisation of former industrial and inner city areas, where much of the contaminated land is located, has also generally been considered to

be desirable not only for economic reasons (as land suitable for redevelopment in inner cities is at a premium) but for aesthetic reasons and the safeguarding of public health (Johnston & Bell, 1980; Pearce, 1981).

After Germany, the UK has the largest share of contaminated land in Europe. Over 20,000 sites of contaminated land are located in prime redevelopment areas which, when redeveloped, would command a high market price. Among the most numerous and widely distributed examples of contaminated land are gas work sites, where town gas was produced from the carbonisation of coal for over 150 years in almost every town and large village in the UK (Wilson & Stevens, 1981).

Government policy, through the Derelict Land Act 1982, is therefore to promote the redevelopment of contaminated land by giving financial incentives which encourage the restoration and re-use of such land. New technology, especially high pressure liquid chromatography (h.p.l.c.) and gas chromatography/mass spectrometry (gc-ms), has made detailed analysis of soils and groundwater possible to an extent hitherto unknown. The technology for cleaning up contamination also exists with the advent of specialist companies e.g., Geokinetics in The Netherlands; Biotreatment in Britain. In 1986 almost half of all new development took place on re-used land, particularly in inner cities and urban areas (Department of the Environment (DOE), 1987).

Another source of land contamination has arisen from the use of harbour and river dredgings as a top soil substitute. Many rivers and harbours need dredging to maintain them in a navigable state. In the UK, dredgings have generally been deposited at sea, but apart from the economic cost, the EC and, within the UK, the Ministry of Agriculture, Fisheries and Food have begun to oppose this practice. The alternative is to deposit the dredgings on land.

Dredgings from the most extensively used rivers are frequently grossly polluted

(Van Driel & Nijssen, 1988). Sediments dredged from the Rhine were found to be contaminated particularly by heavy metals, oil and chlorinated hydrocarbons (Adriaan Volker Dredging Co., 1978). Despite the fact that contamination problems exist, material dredged from harbours and rivers in industrial areas have commonly been used for productive land use (20 ports surveyed in 6 European countries), especially in The Netherlands (Herms *et al.*, 1988; Van Driel & Nijssen, 1988). Ripened dredged material (i.e. the transformation from reduced structureless mud to a more oxidised and drained soil (Bramley & Rimmer, 1988), can be classified as one of the best soils for agricultural use due to the high amount of organic matter and nutrients it contains (De Nekker & D`Angremond, 1976), though Smilde *et al.*, (1982) considered as unfit for human consumption, the produce grown on the harbour-dredged material they investigated due to its content of cadmium.

1.2 History of metal contamination

During the 19th century, Britain was a major source of refined metals, particularly zinc, copper, tin and lead compounds (Davies & White, 1981). Inefficient production, lack of legislation relating to the disposal of wastes, erosion and flooding has led to widespread contamination (Thornton, 1981). An estimated 4000km^2 of agricultural land in England and Wales has been contaminated in varying degrees by past mining and smelting activities (Thornton & Webb, 1975). Contaminants include one or more of the metals Cu, Pb, Zn, Cd and As. The repeated applications of sewage sludge, [0.5 million tonnes of sewage sludge dry matter is spread annually in the UK on agricultural land (DOE, 1981)], containing large quantities of potentially phytotoxic metals, over a long period of time, has also resulted in metal contamination of soils (Williams, 1981).

1.3 History of coal carbonisation

The history and development of the coke and gas industry has been very well documented and reviewed (Coke Manufacturers Association, 1936; British Petroleum, 1972; Key, 1956; Wilson & Stevens, 1981; Environmental Resources Ltd., 1987) and only a brief summary is given here.

Coal carbonisation sites are among the most numerous and the most hazardous of contaminated sites, largely because of the variety and toxicity of the byproducts of coal carbonisation, (Fig. 1) (Wilson & Stevens, 1981; Dean & Goldby, 1983; Smith & Ellis, 1986). Coke, produced by coal carbonisation, has been used for the smelting of iron and steel for centuries. In 1877, there were more than 14,000 beehive ovens in County Durham alone, producing more than four million tons of coal annually. A peak of about 30 million tonnes per annum in Great Britain was reached in 1956, declining gradually with the contraction of the iron and steel industry (Reid, 1972).

Gas was first used for street lighting in 1809. With the advent of electric lighting in 1883 town gas was no longer required for illumination. This led to research into new areas for gas usage and provided the stimulus for town gas to be used for heating and cooking, to the extent that in the 1930`s virtually every town and large village in Britain, had its own gas works (Wilson & Stevens, 1981). By the 1950`s and 60`s the manufacture of gas from coal carbonisation was superceded by the cracking of petroleum and this, in its turn, by the distribution of natural gas from the North Sea in the 1970`s.

Although the process to produce gas was essentially the same for the production of coke or gas, the yield of gas, tar or coke could be optimised depending on the temperature to which the coal was heated (Table 1). Fundamentally, the process

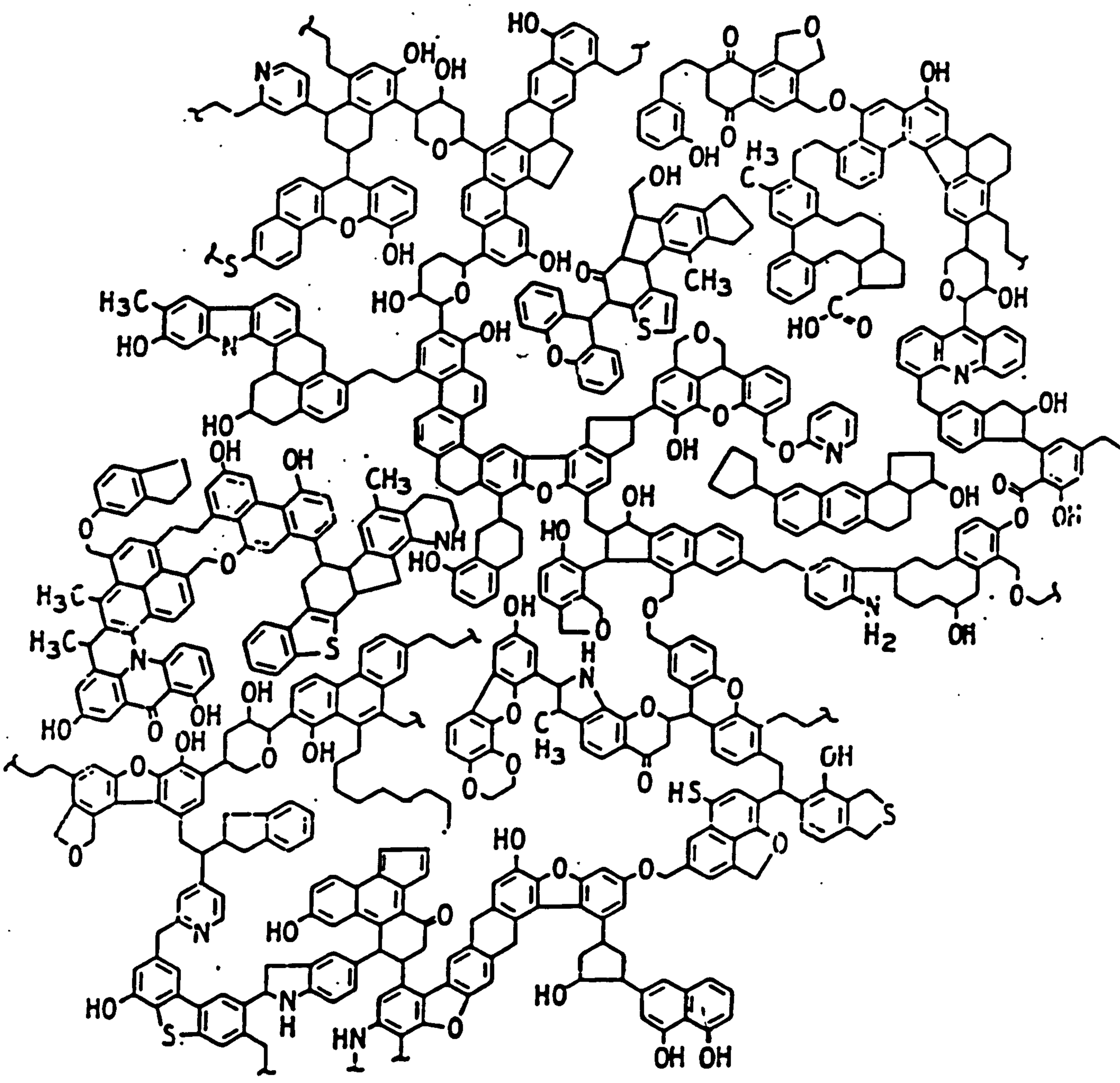


Figure 1. ***Model of Bituminous coal*** (from Shin, 1984).

Table 1. Comparative yield for high and low temperature coal carbonisation.

	low temperature	high temperature
gas produced (m^3/tonne coal)	111	334
coke (% coal weight)	72	65
volatile matter in coal (%w/w)	10	2
tar (litres/tonne coal)	90	54
crude benzole (litres/tonne coal)	18	13
ammonia (kg/tonne coal)	4	8
calorific value of gas (BTU/m^3)	24	14

From: Environmental Resources Ltd., 1987, p8.

consisted of heating coal in vertical or horizontal closed retorts in an oxygen-deficient atmosphere during which about one third of the mass of the coal was converted to gaseous and liquid products (leaving behind a solid coke)(Fig. 2). The crude gas, contaminated with a range of substances such as tar, ammonia, cyanides and hydrogen sulphide, was collected in a hydraulic main where water or ammoniacal liquor circulated. The tars, phenols and ammonia condensed in the circulating liquor and were finally collected in an underground tar/liquor well. The crude gas was purified further in a series of stages. A condenser removed further ammonia, and, residual tar particles were removed by wet scrubbing or electrostatic precipitation. Remaining ammonia was removed in another wet scrubbing step and naphthalene was sometimes recovered by washing with oil. H_2S and HCN were absorbed in beds of ferric oxide. This refining was responsible for many of the residual contamination problems of (now derelict) gas works because technical or economic factors often prevented recycling of the treatment media which were thus discarded when spent (Johnston & Bell, 1980). The main by-products of gas production were: coke, tar, benzole, ammoniacal liquor and spent oxide (DOE, 1977). These were all very valuable commodities and were often sold or processed further on-site (Wilson & Stevens, 1981).

Contamination occurred in a number of ways - spillage, leakage or deliberate dumping of the residues on-site or on adjacent land. A wide range of contaminants are usually present on gas, coking or tar works sites, the most important being coal tar itself - an extremely complex mixture, (its composition varying with its origin and the manufacturing conditions during coal generation), consisting of benzene and alkylated benzenes; phenol and methylated phenols; polyhydric phenols; and polyaromatic hydrocarbons (PAHs) (eg. naphthalene, anthracene, phenanthrene, etc.) (DOE, 1977).

Polyaromatic hydrocarbons (PAHs) are a group of environmental contaminants

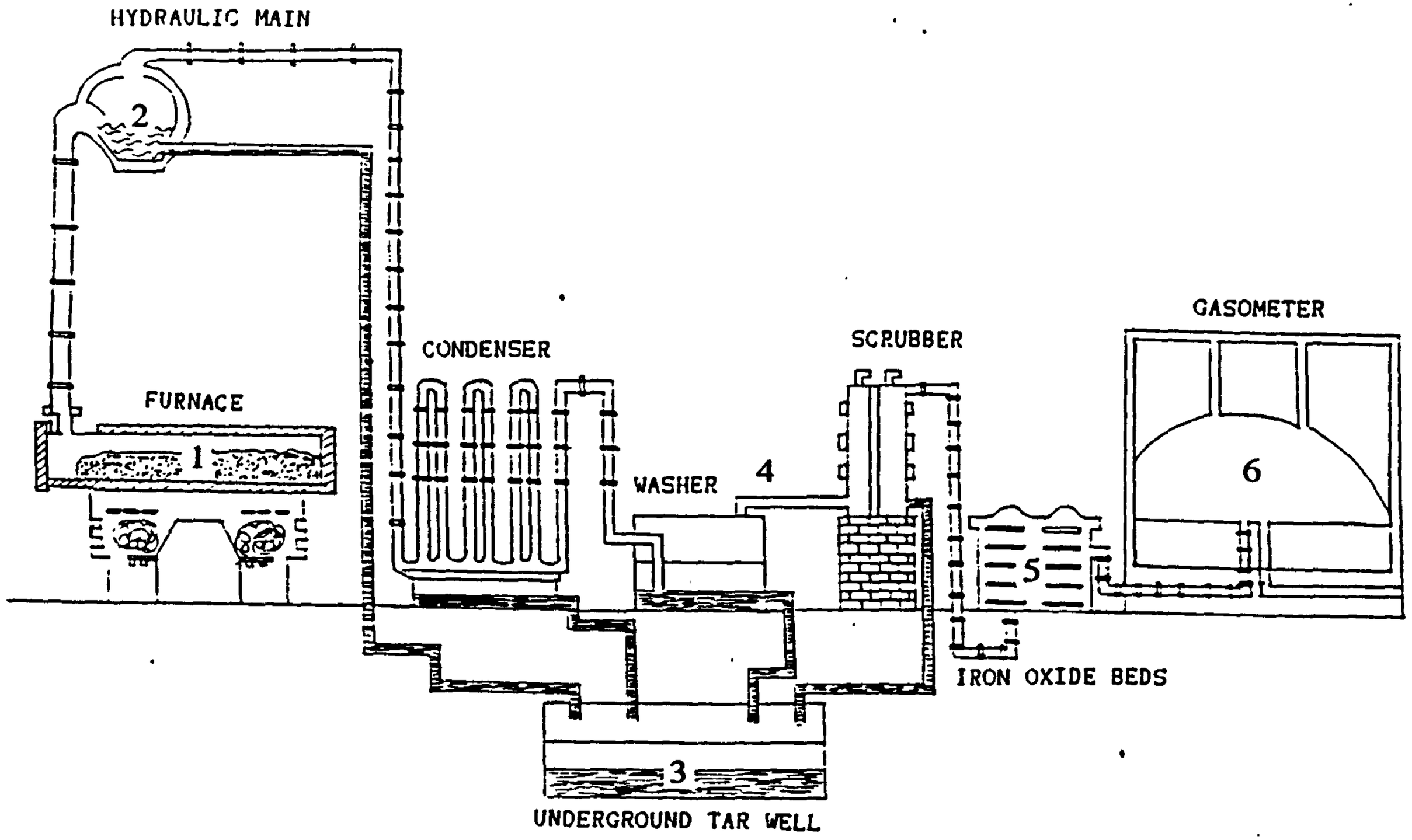


Figure 2. Coal carbonisation works.
Coal was heated in closed retorts (1). The gases released rose to the hydraulic main (2) where phenols, tars and ammonia present in the gas collected in the circulating water or ammoniacal liquor, collecting in the underground tar well (3). The crude gas was purified by passing through a series of condensers, washers and scrubbers (4). Finally the gas was passed over beds of iron oxide (5) to remove H_2S and HCN before storage in a gasometer (6).

classed as priority pollutants by the European Commission and the US Environmental Protection Agency, as many of them are known to be mutagenic and carcinogenic. They originate primarily from the combustion of natural vegetation or fossil fuels which release and disperse PAHs whenever they are burnt (Edwards, 1983). Some aqueous effluent from various coal-based industrial processes have been found to contain 0.05-290μg/l of benzo(a)pyrene, a known carcinogen (Salvesen, 1984). This compares with the Environmental Protection Agency (EPA) levels for drinking water of less than 0.2μg/l collectively for six PAH`s (Jones, 1988).

1.4 Legislation relating to contaminated land

The NATO-CCMS (Committee on Challenges of Modern Society) pilot study group on contaminated land adopted the following definition of contaminated land, "Land that contains substances that, when present in sufficient quantities or concentrations, are likely to cause harm to man, the environment or on occasions to other targets" (Smith, 1984). The legislation that surrounds the need for remediation of such land, however, varies significantly from country to country.

1.4.1 The UK situation

The legal framework relating to contaminated land in Britain has been fragmentary and piecemeal with legislation tackled from a variety of disciplines (Table 2). It is important to realise that up until about 1972, there was little or no effective legislation directly concerning the deposit of waste on land. Even now, loopholes exist that allow `fly-tipping`, so the potential for chemical contamination still exists (Hillman, 1984). Up to the 1970`s the disposal of waste material was an unregulated

Table 2. Legislation relating to contaminated land.

Public Health Act 1936
Town and Country Planning Act 1971
Deposit of Poisonous Waste Act 1972
Control of Pollution Act 1974
Health and Safety at Work Act 1974
Derelict Land Act 1982
Notification of Installations Handling Hazardous Substances Regulations 1982
Control of Industrial Major Accident Hazard Regulations 1984
Reporting of Injuries, Diseases and Dangerous Occurrences Regulations 1985
Building Regulations 1985
Control of Substances Hazardous to Health Regulations 1988
Environmental Impact Assessment 1988
Environmental Protection Act 1990

localised operation throughout the UK. The disposal of industrial and other waste substances was undertaken by the manufacturer (often simply dumping waste on site) or by a local haulier, usually to the nearest of one of the many thousands of possible sites in the UK. Sites were selected because of convenience rather than suitability, with no distinction between industrial and domestic wastes. The main controls on the redevelopment of contaminated land lay with Local Authorities through their planning systems. Controls and conditions could be put on the use of the land and remedial work could be specified but was rarely initiated. An incident regarding the concealment of cyanide wastes in 1972, was the fore-runner to the Deposit of Poisonous Waste Act (1972) which was introduced to tighten up legislative control, by penalising the deposition on land of poisonous, noxious or polluting wastes. This was followed by the Control of Pollution Act, 1974. Together the acts increased professional standards within the waste management industry by setting a `duty of care` on the Waste Disposal Authorities to ensure arrangements existed for waste arising or being imported into its area and the licensing of fully engineered and controlled landfill sites (Hawkins, 1980).

In response to the general problem of land contamination, the Interdepartmental Committee on the Redevelopment of Contaminated Land (ICRCL) was set up in 1976, with representatives from the Department of the Environment, the Scottish Development Department, Welsh Office, Department of Health and Social Security, Health and Safety Executive and the Ministry of Agriculture, Fisheries and Food. Its terms of reference were "to develop and co-ordinate advice and guidance on human health hazards arising from the re-use of contaminated land; to develop and co-ordinate advice regarding remedial measures; to make such advice available to local authorities; to advise the Department of the Environment (DOE) as to the appropriateness of the methods selected by local authorities and; to identify research needs" (Department of the

Environment, 1989). The ICRCL through the DOE has subsequently:

1) supported research through the Government`s Environmental Protection Scheme.

2) funded the Derelict Land Grant Scheme (DLG), 1981, to restore derelict and contaminated land in England. Since then, the City Grant, 1988 has been introduced within 57 priority areas, to replace the Derelict Land Grant. DLG remains a significant fund source out with those 57 priority areas (Ironside, 1989).

3) played an important part in the development of national standards, through the British Standards Institute (BSI), though this has been overtaken by the International Standards Organisation (ISO).

4) been involved in the NATO/CCMS pilot studies on contaminated land. The initial study reviewed technologies available for dealing with contaminated sites and, in the 5-year study, 27 projects demonstrating soil clean-up techniques have been reviewed and evaluated (Smith, 1984, 1986a & 1989).

Since its inception in 1976, the ICRCL has played an important part in providing and co-ordinating informed advice on the redevelopment of contaminated land and has published a number of papers on the subject. The ICRCL have introduced guidelines on levels of contaminants in soil (Tables 3 and 4). There are no guidelines for pesticides, polychlorinated biphenyls (PCBs), chlorinated aliphatics, mineral oil, iron, barium and ammonium though Kelly, (1981) has produced a more comprehensive set of guidelines for contaminated soils (Table 5).

The ICRCL`s guidelines are in the form of `trigger values` which differentiate three zones of soil contamination. The two trigger values are defined as a) threshold trigger value, below which " the site may be taken as uncontaminated" and no remedial action is required, and, b) action trigger value, above which some form of remedial action is required, or the form of development changed. These two trigger values define

Table 3: Tentative "trigger concentrations" for contaminants associated with former coal carbonization sites (from DOE, 1987).

Contaminant	Proposed Uses	Trigger Concentrations (mg/kg air-dried soil) Threshold	Action
Polyaromatic hydrocarbons (1,2)	Domestic gardens, allotments, play areas.	50	500
	Landscaped, buildings, hard cover.	1000	10000
Phenols	Domestic gardens, allotments.	5	200
	Landscaped, buildings, hard cover.	5	1000
Free cyanide	Domestic gardens, allotments, landscaped.	25	500
	Buildings, hard cover.	100	500
Complex cyanides	Domestic gardens, allotments.	250	1000
	Landscaped.	250	5000
	Buildings, hard cover.	250	NL
Thiocyanate (2)	All proposed uses.	50	NL
Sulphate	Domestic gardens, allotments, landscaped.	2000	10000
	Buildings (3).	2000 (3)	50000 (3)
	Hard cover.	2000	NL
Sulphide	All proposed uses.	250	1000
Sulphur	All proposed uses.	5000	20000
Acidity (pH <)	Domestic gardens, allotments, landscaped.	pH 5	pH 3
	Buildings, hard cover.	NL	NL

1. Used here as a marker for coal tar, not analytical reasons.
2. See `Problems Arising from the Redevelopment of Gas Works and Similar Sites` for details of analytical methods.
3. See also BRE Digest 250: Concrete in sulphate-bearing soils and groundwater.

NL: No limit set as the contaminant does not pose a particular hazard for this use.

Table 4. Tentative Trigger Concentrations for selected inorganic contaminants (from DOE, 1987).

Contaminants	Planned Uses	Trigger concentrations (mg/kg air-dried soil) threshold	action
Group A: Contaminants which may pose hazards to health			
Arsenic	Domestic gardens, allotments.	10	*
	Parks, playing fields, open space.	40	*
Cadmium	Domestic gardens, allotments.	3	*
	Parks, playing fields, open space.	15	*
Chromium (hexavalent)	Domestic gardens, allotments,		
	parks, playing fields, open space.	25	*
Chromium (total)	Domestic gardens, allotments.	600	*
	Parks, playing fields, open space.	1,000	*
Lead	Domestic gardens, allotments.	500	*
	Parks, playing fields, open space.	2,000	*
Mercury	Domestic gardens, allotments.	1	*
	Parks, playing fields, open space.	20	*
Selenium	Domestic gardens, allotments.	3	*
	Parks, playing fields, open space.	6	*
Group B: contaminants which are phytotoxic but not normally hazards to health			
Boron (water-soluble)	Any uses where plants are to be grown	3	*
Copper (4,5)	Any uses where plants are to be grown	130	*
Nickel (4,5)	Any uses where plants are to be grown	70	*
Zinc (4,5)	Any uses where plants are to be grown	300	*

* Action concentrations will be specified in the next edition of ICRCL 59/83

4. Total concentrations (extractable by $HNO_3/HClO_4$)

5. The phytotoxic effects of copper, nickel and zinc may be additive. The trigger values given here are those applicable to the `worst-case` : phytotoxic effects may occur at these concentrations in acid, sandy soils. In neutral or alkaline soils phytotoxic effects are unlikely at these concentrations.

Table 5. Guidelines for contaminated soils.
Suggested range of values (mg/kg air-dried soils) (Kelly, 1981).

Parameter	Typical values for uncontamin`d soils	Slight contamination	Contaminated	Heavy contamination	Unusually heavy contamin.
pH (acid)	6-7	5-6	4-5	2-4	<2
pH (alk)	7-8	8-9	9-10	10-12	>12
Antimony	0-30	30-50	50-100	100-500	>500
Arsenic	0-30	30-50	50-100	100-500	>500
Cadmium	0-1	1-3	3-10	10-50	>50
Chromium	0-100	100-200	200-500	500-2500	>2500
Copper (avail)	0-100	100-200	200-500	500-2500	>2500
Lead	0-500	500-1000	1000-2000	2000-1.0%	>1.0%
Lead (avail)	0-200	200-500	500-1000	1000-5000	>5000
Mercury	0-1	1-3	3-10	10-50	>50
Nickel (avail)	0-20	20-50	50-200	200-1000	>1000
Zinc (avail)	0-250	250-500	500-1000	1000-5000	>5000
Zinc equiv.	0-250	250-500	500-2000	2000-1.0%	>1.0%
Boron (avail)	0-2	2-5	5-50	50-250	>250
Selenium	0-1	1-3	3-10	10-50	>50
Barium	0-500	500-1000	1000-2000	2000-1.0%	>1.0%
Beryllium	0-5	5-10	10-20	20-50	>50
Manganese	0-500	500-1000	1000-2000	2000-1.0%	>1.0%
Vanadium	0-100	100-200	200-500	500-2500	>2500
Magnesium	0-500	500-1000	1000-2000	2000-1.0%	>1.0%
Sulphate	0-2000	2000-5000	5000-1.0%	1.0-5.0%	>5.0%
Sulphur (free)	0-100	100-500	500-1000	1000-5000	>5000
Sulphide	0-10	10-20	20-100	100-500	>500
Cyanide (free)	0-1	1-5	5-50	50-100	>100
Cyanide (total)	0-5	5-25	25-250	250-500	>500
Ferric cyanide	0-100	100-500	500-1000	1000-5000	>5000
Thiocyanate	0-10	10-50	50-100	100-500	>500
Coal Tar	0-500	500-1000	1000-2000	2000-1.0%	>1.0%
Phenol	0-1	2-5	5-50	50-250	>250
Toluene ext.	0-5000	5000-1.0%	1.0-5.0%	5.0-25.0%	>25.0%
Cyclohexane ext.	0-2000	2000-5000	5000-2.0%	2.0-10.0%	>10.0%

three zones of contamination, that is, 1) the uncontaminated zone - below the threshold trigger value; 2) the undesirable zone - the area between the threshold trigger value and the action trigger value, and, 3) the action zone - above the action trigger value (Environmental Resources Ltd., 1987). The report draws attention to the phrase "treat as uncontaminated" as with certain end uses (e.g. car parks) it may be possible to treat the site as uncontaminated even if concentrations of contaminants are such as to indicate contamination. Thus the DOE (1987) paper and Beckett & Simms (1986) from the Central Directorate on Environment Protection suggest making the development fit the site rather than one which is concerned with using remedial measures where necessary to make the site fit the development. Likewise in the `undesirable zone` interpretation of the contamination levels of pollutant requiring remedial action is dependent on cost, and the analytical results. The DOE`s ICRCL 59/83 Report (2nd Ed., 1987) states, "even though the threshold value is exceeded this does not automatically mean that the risk of the hazard is significant; merely that there is a need to consider whether the presence of the contaminant justifies taking remedial action". Above the action trigger value some remedial work will be required though, `its nature and extent will depend on local circumstances` (Environmental Resources Ltd., 1987). The government emphasise that action, suggested when threshold levels are exceeded, is not mandatory.

The trigger concentrations are for sites about to be developed and are not applicable to sites already in use or in the process of being redeveloped (DOE, 1987). This difference is probably forced by financial constraints, as there can be substantial difference in costs for treatments which tackle environmentally-unacceptable conditions and those tackling only unacceptable conditions for redevelopment. This pragmatic approach that the DOE and the government has adopted is cheaper and more cost effective than, though at variance with, that adopted in several other countries, notable

the US and The Netherlands who both have major national programmes to clean-up contaminated land.

The House of Commons Environmental Committee (DOE, 1990) criticised the Government`s stance towards land blighted by past industrial use and stated that Britain`s methods of cleaning up land are often technically inferior to Dutch and West German practices. MPs expressed concern that Britain has made relatively little use of biotechnological techniques to clean contaminated land.

The government have accepted the ICRCL guidelines on threshold and action trigger values with the caveat that as the concept can vary, threshold levels should be higher where background levels are higher (Society of Chemical Industry (SCI) conference, 1989). This view has been the subject of much dissent, particularly in Somerset and Cornwall where background levels of some metals, e.g. Cd and Zn, are particularly high (Thornton, 1981; Simms & Beckett, 1987).

The Environmental Protection Act 1990 came into force in April 1991 in England and Wales and April 1992 in Scotland. Through it, the government have introduced a system of Integrated Pollution Control (IPC). This will be enforced by her Majesty`s Inspectorate of Pollution (HMIP) in England and Wales, while in Scotland this responsibility is split between HM Industrial Pollution Inspectorate and The River Purification Authority. Their remit will be the setting of standards and controlling all major emissions to air, land and water from the most polluting industrial processes by applying BATNEEC (best available techniques not entailing excessive cost) to limit pollution. The Act introduces tighter environmental controls, stiffer penalties and strengthens the power of HMIP to control pollution (Environmental Protection Act, 1990). Potential polluters will have to pay for this control system. HMIP will recover the bulk of the costs of operating IPC by charging for discharge authorisation (Murley,

1992).

The government has ruled out a full study of the extent of Britain`s land contamination as `prohibitively expensive`. Instead, under the Act, local authorities are required to produce public registers of land "subject to contamination" in their area. Such registers are expected to be compiled mainly from historic archives. According to some estimates this could cover some 100,000 former industrial sites and may include hundreds of thousands of homes build on them (Pearce, 1992). Unfortunately there is no system of taking remediated contaminated land off the register This has led to (an understandable) caution among would-be developers unwilling to have the stigma of contaminated land where, even after clean-up, there is no means of removing it from the register. Due to the fierce criticism that the register would cause a wholesale planning blight in the inner cities, the Government have withdrawn plans to register all potentially contaminated sites and in August 1992, issued revised proposals which called for only 10-15% of polluted sites to be put on registers immediately, the remainder to be registered `in due course`. A duty of care is equally placed on all those involved in dealing with waste from its generation to its disposal. The newly formed HMIP has a difficult task ahead as there is still a lack of cohesion between various branches of the law (Tromans, 1987) and with a policy of BATNEEC the setting and tightening of standards will be difficult, although the realisation that environmental pollution has to tackled as a whole is an important step towards a comprehensive environmental policy.

1.4.2 The situation in other countries

Approaches to developing and establishing a programme for assessment and the clean-up of contaminated land varies widely between countries. In a few countries there is explicit uniform national guidance (The Netherlands, US, Germany, Austria); in others, each regional authority has to plan its own reclamation programme (Italy, Switzerland); in yet others, improvement of legislation for environmental control has just been initiated (Czech & Slovak Federal Republic) while in yet other countries indiscriminate dumping of waste materials is practised (Yemen Arab Republic; India). Article 130R of the Single European Act is aimed at improving environmental quality, protecting human health and managing natural resources. The Commission will be determining standards and establishing maximum permissible levels for `injurious` contaminants (Pandolfi, 1989). In addition European environment ministers met in June 1991 and decided to produce a report that will facilitate the development of an environmental programme for Europe which will identify priorities for the repair and restoration of existing environmental damage and the prevention of future problems (Bourdeau, 1991).

The first country to establish a national comprehensive set of criteria for contaminated land was The Netherlands. In The Netherlands, land is traditionally regarded as a scarce and precious resource. Population density is high, and consequently the demand for land, is great. Thus policies which would permit a considerable amount of permanently contaminated land, unsuitable for certain types of human use, are not acceptable. Also, in many parts of The Netherlands, the ground water level is high, so that in the industrial areas where most of the contaminated land is situated, ground water contamination would almost invariably result. There is a genuine fear of such contamination spreading and polluting ground water drinking supplies

(Keuzenkamp, 1990). Generic criteria (ABC levels) for evaluating the significance of pollution enacted in The Soil Clean-up Interim Act (1984) often referred to the `Dutch List` (consisting of 12 metals, 7 inorganics, 25 organics and 7 others) has been adopted by the Scandinavian countries, Canada and Germany. The Dutch government aims to restore soil quality at the site and to purify soil and ground water by incorporating the Soil Clean-up Act (1988) into an expanded general law for the protection of the soil (National Environmental Policy Plan, 1990). The central concept of environmental policy is `sustainable development`. This concept has been developed by The Brundtland Commission, (1987). It emphasizes the need to restore the multifunctionality of the land, so ideally, current use of land would keep the options open for other uses in the future (Keuzenkamp, 1990). Therefore only permanent and definitive solutions are acceptable in cleaning up contamination. Initially in The Netherlands, it was the central government that financed such projects, though they are now trying to recover the costs *via* civil jurisdiction and by encouraging industries causing contamination to take the initiative and clean it up (Eikelboom & Von Meijenfeldt, 1986; Von Meijenfeldt & Schippers, 1990). Evaluation of the soil protection policy for the last 10 years has reaffirmed environmental protection but has shifted the emphasis on the `polluter pays` and integrates clean-up of existing soil with prevention of new contamination. New legal provisions will compel owners of industries with the highest risk of polluting the soil (25,000 sites) to investigate the state of the soil and take remedial action if serious soil contamination is present and to take adequate protective measures to prevent further contamination (Gravesteyn, 1990; Keuzenkamp *et al.*, 1990).

1.5 Land Reclamation

Certain types of site in the UK have been found to be particularly likely to be contaminated (DOE, 1987) (Table 6).

The remedial measures used for a particular site will depend on the nature, extent and toxicity of the contaminant, the type of soil and the form in which the contaminant is present in the soil, together with financial constraints, time available for redevelopment and the proposed end use. The interaction of the principal factors in treatment selection has been illustrated by Barry, 1982 (Fig. 3).

In practice many methods available will not be suitable for a particular site, and will tend to be expensive or have other drawbacks; therefore a combination of methods is often used. Obviously the more sensitive the proposed end-use (e.g. housing) the more extensive will be the need for investigation and remedial measures (DOE, 1987).

Recently the redevelopment of land contaminated with industrial waste has attained a higher profile for the reasons mentioned above and examples of treatment have been well documented (Assink & Van den Brink, 1986; Wolf *et al.*, 1988; SCI conference, 1989; Arendt *et al.*, 1990). Several excellent review articles of the possible techniques for remediation of contaminated soil and groundwater have appeared (Barry, 1982; Smith, 1985; Cairney, 1986c; Jessberger, 1986; Mischgofsky & Kabos, 1988; Morgan & Watkinson, 1989 and 1989a). The account outlined below summaries the main methods.

Britain together with most other countries has traditionally used excavation, cover and barrier methods for contaminated wastes, with provision for treatment of groundwater and leachate. In Britain, however, treatment of groundwater has been largely neglected as the majority of drinking water derives from the uplands and from reservoirs. This neglect will have to be remedied when the EC directive to protect

Table 6. Sites likely to show significant contamination.

Landfill sites
Gasworks or other coal combustion sites
Sewage works and sewage farms
Scrap yards
Railway land, particularly railway sidings
Oil and fuel storage and distribution depots
Metal mines, smelters, foundries, steel works and metal finishing works
Chemical works
Munition production and testing sites
Asbestos works
Tanneries
Paper and printing works
Industries making or using wood preservatives.

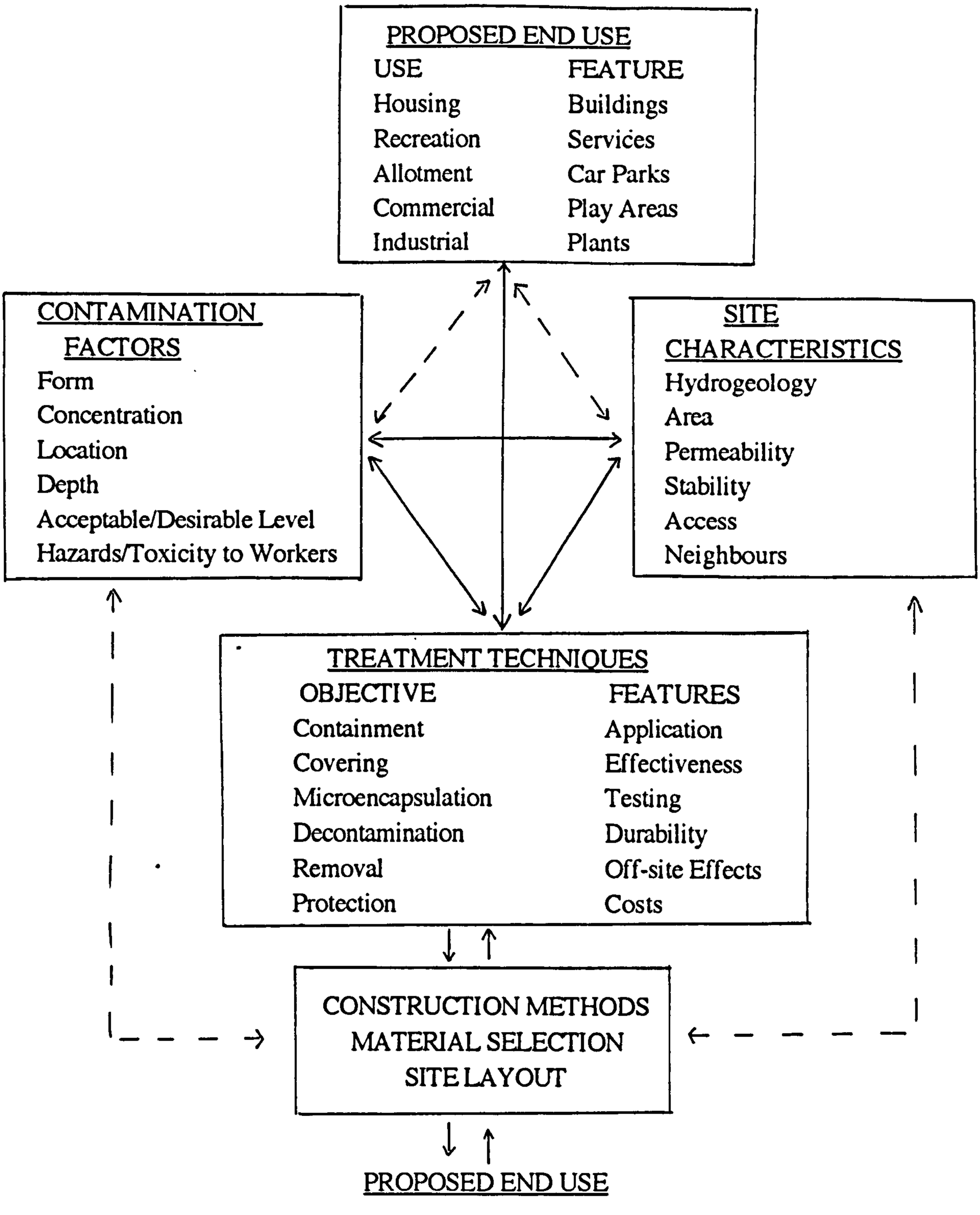


Figure 3. The interaction of principal factors in treatment selection (from Barry, 1982).

groundwater (Offical Journal of European Communities, 1979) comes into force because although only 25-30% of our water supplies are taken from groundwater (Smith, 1986b), once aquifers are contaminated they are very difficult and expensive to clean up. It is noticeable that Denmark, Germany and, in particular The Netherlands have a more far-sighted approach.

With increasing awareness of the finiteness of available land, attention is concentrating on the optimisation of isolation methods and existing techniques for treating excavated soil and on the improvement and development of *in situ* techniques (Mischgofsky & Kabos, 1988). The Royal Commission on Environmental Pollution (1985/6) summed up environmental problems regarding pollution in stating:

"Unless an industrial pollutant can be eliminated as opposed to being transformed, it must be disposed of somewhere" (5th report, para. 265).

1.5.1 Remedial measures

The remedial measures used to clean up contaminated soil can be broadly divided into 2 categories (Hitchens & Smith, 1983).

A) The first category involves methods aimed at preventing or restricting the dispersion of the contamination to the immediate surroundings. Excavation of contaminated soil to another site is applicable to most contaminants on most sites, but costs are high if volumes large and the problem is not solved but transferred to another site.

Traditionally reclamation of land contaminated with heavy metals has involved covering metal contaminated soils with clean material. Traditionally this has been topsoil, but cost and the possibility of root penetration and the upward movement of metal ions has encouraged the use of other materials as barrier layers (Jones *et al.*,

1981). Barriers of porous subsoil (Gemmell, 1974) and shale (Johnson *et al.*, 1977) have been used over severely contaminated sites such as chromate smelter waste and Pb/Zn mining waste. As with all barriers these methods of overcoming soil toxicity, though successful in the short term, require constant monitoring as chemical and biological soil conditions change with time.

Covering contamination with horizontal barriers, usually soil, has been the most common method of reclamation undertaken in the UK (DOE, 1987), especially for former gas works, and waste disposal tips (Cairney, 1986a). Britain has rarely attempted methods used in other countries such as large scale removal or on-site treatment of contaminated soil and groundwater. When the 300 ha Consett steelworks, Durham, closed in 1982 the government spent £8 million largely to bury the contaminated soil. By contrast at a gas works in Utrecht, The Netherlands, the authorities spent £100 million to clean up 8 ha (Pearce, 1992). A number of materials are used as horizontal barriers. These include: soil, soil bound with cement, sewage sludge, fly ash or plastic sheeting. The horizontal barrier has a number of functions including, 1) the protection of the contaminated soil from rain thus reducing percolation through to groundwater, 2) the avoidance of capillary rise of contaminants, 3) the control of erosion, 4) protection of the atmosphere from harmful gases and 5) the support of vegetation (Cairney, 1986a). *Vertical barrier systems* are designed to reduce the migration and diffusion of water soluble contaminants. Thus the barrier has to be impermeable and as stable against aggressive contaminants as possible. Sheet steel walls, high density polyethylene, grouting (where cement/clay mixtures are pressure injected into the ground) and slurry walls (trench filled with concrete or bentonite) have been used.

Though the financial aspects of this method make it appear attractive, engineering constraints and the integrity of treatment components are critically related to

soil and water conditions so that proper consideration of these features has to be given before it is used, as noted in Cairney's very extensive articles (1984; 1986; 1986a & 1986b). In general, barrier systems have a finite life, losing their effectiveness with time and requiring regular monitoring, maintenance and renewal (Cairney, 1986b).

In practice, isolation by barrier is preferred in Britain. The government suggest (DOE, 1983, 1987) that site development can be adjusted so that the most badly contaminated areas are located beneath permanent hard cover (roads, etc), leaving less contaminated parts of the site for the main buildings or for gardens and amenity areas (DOE, 1987).

B) The second category of remedial measures includes those aimed at destroying or removing the contamination; i.e. to provide a final solution. The simplest method of remedial action has been *the dilution of contaminated soil* with clean soil. This can only be successful where area is large, contamination low and the range of land uses are wide enough to permit flexibility in allocating them. On-site processing (i.e. cleaning after excavation) involves excavation of the soil and decontamination of the soil usually by either thermal treatment or extraction. Thermal methods include steam stripping, (contaminants are released via the steam); heating soil up to 600°C to evaporate the volatiles, and incineration of soil (heating up to 1200°C). In all cases the steam and gases released have to be treated. Thermal methods have been successful for all types of vaporizable material, e.g. aromatic and hydrogenated hydrocarbons, PAHs and mineral oils (Hilberts *et al.*, 1986; James & Sanning, 1989) but are chiefly directed at chlorinated hydrocarbons with the aim of preventing dioxin formation. Thermal treatment can cause changes in the physical and chemical characteristics of the soil (e.g. loss of organic matter, increase in pH, increase in amount of extractable cations, damage of the clay fraction, decrease of water-holding capacity, fixation of

plant-available phosphates (Campino *et al.*, 1990) and reduction in cation exchange capacity (CEC) (Goetz & Claussen, 1990). Soil vitrification, heating soil *in situ* by electric current, to a temperature of 2000°C, destroying more than 99.9% of the organics and immobilising more than 90% of the metals in the glass has recently been reported (Van Kasteren, 1990).

Soil venting, a method of remediating hydrocarbon-contaminated soils by use of a vacuum well to induce vapour flow through the subsurface, is still in the experimental stages but may be useful way of remediating gasoline spills (Johnson *et al.*,1990).

Versluijs *et al.*, (1988) and Van Gestel *et al.*, (1988) examined a number of clean-up methods available for metal-contaminated soils, including thermal heating (up to 600°C), acid extraction at pH 3, extraction with nitrilotriacetate chelater and fractionation with dilute alkali. They concluded that although total amounts of heavy metals in soils were reduced, the remaining metals demonstrated an increased mobility and therefore bioavailability for soil living organisms. Although this did not usually lead to an increased concentration of metals in plants or worms, levels of cadmium and lead were sometimes higher in plants and invertebrates living in 'cleaned' soils than those living in previously polluted soils.

Contaminants can be extracted from soil by: physical or biological adsorption; particle size by means of centrifugation; sieving or electrostatic precipitation of soil particles; washing with water, aqueous solutions, foam or solvents. Biosorption (Gadd, 1990) of metals by microorganisms or their products, e.g. extracellular polymers, has been used successfully to remove low levels of metals from waste waters (Kasan & Baecker, 1989; Venobacher, 1990). Specific companies have been formed to develop a market for biosorption (Brierley *et al.*, 1986) by pelletized non-

living microbial cell mass as a bioabsorbant for Cu, Cd, Zn Pb and Ag from a dilute solution with a 99% success level. This could be useful as a final `polishing system` for other methods.

Contaminants are frequently held on to the clay particles or organic humus in the soil. By using preferential extraction based on particle size, the clean soil can be removed leaving less contaminated soil to treat (Assink,1986). Water, alkalis, acids, solvents or foam can also be used to extract soil contaminants but potentially large amounts of the now contaminated water or solvent have then be treated. Couillard *et al.*, (1991) used a hot water process (90°C + surface active agent) for extracting bitumen from 'tar' sands. Steam stripping and soil washing has been used in the UK quite successfully (SCI conference, 1989). The use of ion exchange resin after an acid wash, successfully removed cadmium from soil in The Netherlands (James & Sanning, 1989) and activated carbon has been used to remove vapours containing volatile organics.

Chemical, biological or physical treatment to destroy or immobilise contaminants can be expensive, but does guarantee a 'once and for all' solution to contamination problems. The Dutch government has chosen on-site soil treatment, at a central processing plant, as its best long term option for dealing with contaminated sites in The Netherlands. In Holland and Germany, the soil is moved to an incinerator or an incinerator is taken to the site. Unfortunately after treatment, such soil is often rendered inert unless it receives the addition of fertiliser and organic matter.

Over the last six years, electroreclamation by electrophoresis, electro-osmosis and electrolysis has been developed by a company in The Netherlands, Geokinetics, to remove heavy metals from soil *in situ* (Lageman *et al.*, 1989). The soil is electrically charged with direct current from an anode and cathode. Different chemical solutions,

constantly circulating around the electrodes, transport 'captured' metals to a purification system above ground. This method achieved a 90% removal of heavy metals when it was used to a depth of 60m on a site in Holland.

1.5.2 Microbial bio-restoration

The technology of using microbial degradative ability for the treatment of organic wastes is not new as microorganisms play an essential role in the decomposition and recycling of animal and plant residues. Soil application for the treatment of human waste was the first engineered method for waste disposal and is centuries old. Biological treatment of coke-work wastes on sites has been adopted in the UK for many years as the most cost-effective means of treating effluents from the coking industry (Catchpole & Stafford, 1977).

Microbial techniques for the treatment of contaminated soil have been called a variety of names - bioreclamation, biorestoration, bioremediation or biotreatment - and have been applied at a number of locations contaminated with organic compounds, especially hydrocarbons. Industrially-polluted land may contain a variety of organic compounds which are amenable to biological degradation, e.g., refinery processing plants polluted with oil and hydrocarbons, or gas works polluted with cyanides, phenols and coal tars. A large number of these compounds have been shown to be biodegradable by bacterial and fungal species already present in the contaminated soil.

Microbial clean-up methods are based on the stimulation of the natural degradation processes in the soil itself by providing optimal environmental conditions either by `landfarming`, *in situ* biorestoration, or the use of on-site bioreactors so that biodegradation can proceed at the maximal sustainable rate. Land farming, or treatment

of the soil surface, is relatively easy but only feasible when contamination is limited to the top 0.5m soil. Soil conditions are set to optimise biodegradation; the soil is usually fertilised and tilled to ensure good mixing and aeration. Site pH may need to be raised to facilitate biodegradation and this can be achieved with the addition of lime. This method has been applied successfully to land contaminated with oil or fuel spills in The Netherlands (De Kreuk, 1986); USA (Raymond *et al.*, 1976; Song *et al.*, 1990) and in West Germany (Ellis *et al.*, 1990). Morgan & Watkinson (1989) confirmed the safety and efficiency of this technology. The Canadian oil industry has been applying oily wastes to land for the last 40-50 years with the complete degradation of the majority of its components (Hosler *et al.*, 1988). Bioremediation of former gas work sites at Doncaster and Blackburn in England and in Germany by Biotreatment Ltd., UK was achieved using the same method but, in this case, addition of a microbial inoculum and covering the soil with polythene to raise the temperature and maintain moisture control, further accelerated biodegradation. Conventional techniques (burial) were used for non-biodegradables, i.e., metals and spent oxides contaminated with complexed cyanides (Bewley, 1986; Bewley *et al.*, 1989). Composting works to the same principle but the soil, mixed with fertiliser and straw or wood chippings to provide organic matter and increase bulk and so aid aeration, is piled into mounds and left to incubate. This has been successful in the treatment of chlorophenol-contaminated soil (Valo & Salkinoja-Salonen, 1986).

Biodegradation of PAHs (e.g. naphthalene, anthracene, phenanthrene, fluorene, fluoranthene, pyrene, benzo(a)pyrene) by both bacteria and fungi have been reported by several authors (Cerniglia & Gibson, 1979; Edwards, 1983). Naphthalene and anthracene were more readily utilised by many soil bacteria than other PAHs (Hosler *et al.*, 1988) though pyrene-degrading bacteria have been isolated by Heitkamp (1988) and Shabad (1967) reported 55-84% degradation of benzo(a)pyrene

within 6-8 days by soil bacteria.

The PAHs are extremely insoluble in water and their degradation rate appears to be a function of this and the number and orientation of condensed rings present in the compound (Wodzinski & Coyle, 1974; Wild *et al.*, 1990). When sludge was added to soil in a controlled land farming experiment, persistence of PAHs increased with the number of condensed rings. The total remaining percentage of 3-ring, 4-ring, 5-ring and 6-ring PAHs was 1.4, 47.4, 78.5 and 78.3% respectively after 1280 days (Bossert *et al.*, 1984). Likewise the work of Barnhart & Meyers (1991) on bioremediation of oil/tar-contaminated soil reported a fall of total PAHs from 3000ppm to 45ppm over the 10-week treatment period, with benzo(a)pyrene being reduced by approximately 65%.

Carcinogenic and mutagenic intermediate products may result from the degradation of some PAHs (Cerniglia & Gibson, 1979; Cripps & Watkinson, 1978) and are probably responsible for the carcinogenicity of the PAHs in question, though Shabad (1967) found benzo(a)pyrene intermediates were less carcinogenic to mice than benzo(a)pyrene itself. Stroh *et al.*, (1990) found no dangerous metabolites were produced during the microbiological degradation of a former gas work-contaminated soil. Currently there are no legal enforceable restrictions specifically aimed at limiting the input of organic contaminants to agricultural soils from sewage sludges and concern has been expressed over the increasing levels of PAHs in soil from the addition of sewage sludge (Wild *et al.*, 1991) and atmospheric pollution (Jones,1988). Losses of PAH concentrations in soil have been monitored and half-lives were found to vary from <2.1 years for naphthalene to 16.5 years for coronene (6-ringed compound). Higher molecular weight compounds were even more recalcitrant.

Where contamination is deeper, enhanced subsurface biodegradation has been used (Fig. 4). The process consists of the stimulation of indigenous bacteria by the

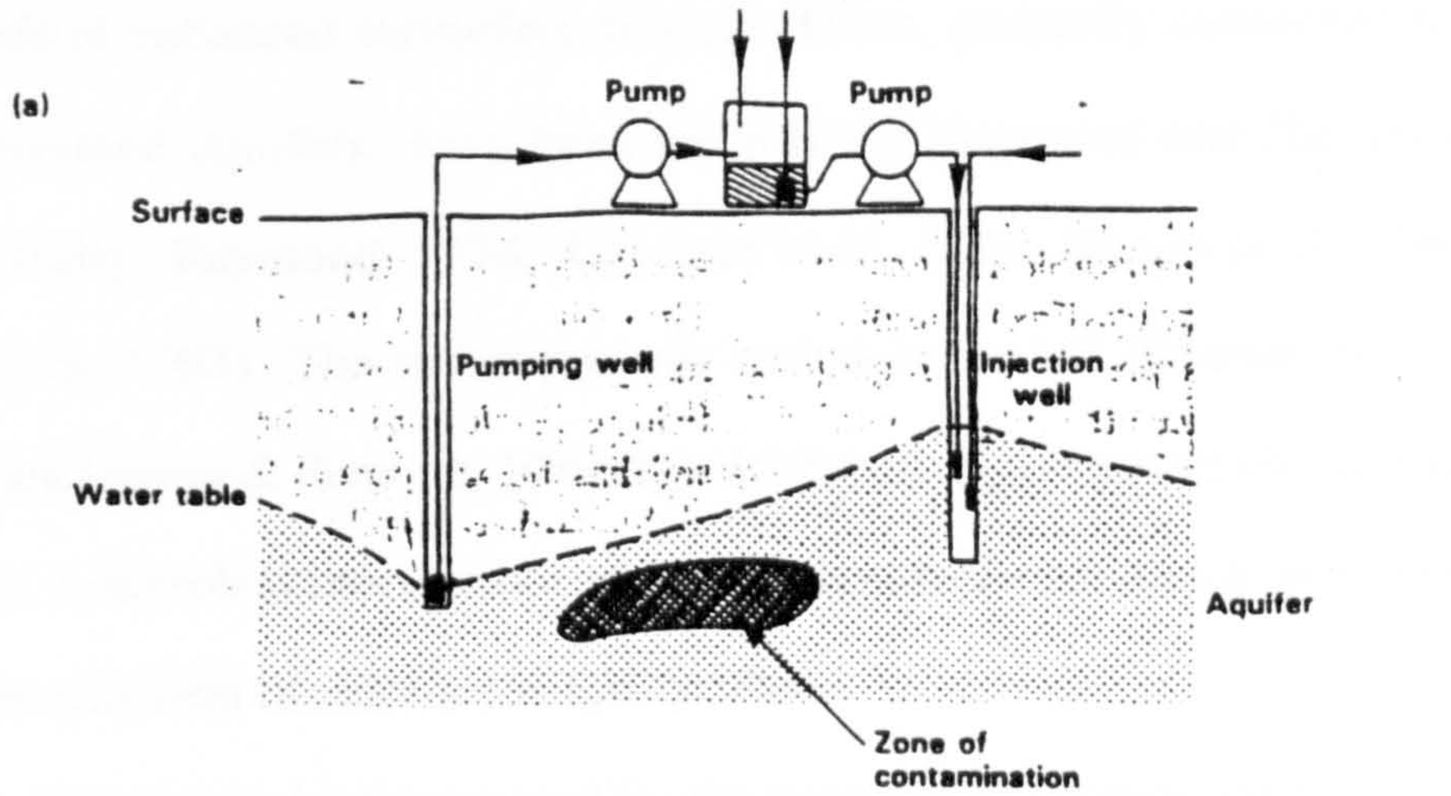


Figure 4. Diagrammatic representation of the principles involved in *in situ* microbial clean-up of the subsurface.

a) Illustrates a location where the aquifer is contaminated and a pumping-injection circulation system permits continuous supply of nutrients and/or oxygen. b) Illustrates a location where nutrient supply to the subsurface is being performed by allowing percolation of nutrients and/or microbes from an irrigation sprayfield (from Morgan & Watkinson, 1989a).

recirculation of groundwater through the contaminated soil; by adding oxygen and nutrients and by venting volatiles. Oil-contaminated aquifers, (Batterman, 1986), and jet fuel/petroleum-contaminated soil and groundwater have been successfully treated in the USA and The Netherlands (James & Sanning, 1989; Payne & Floyd, 1990). Two methods of enhanced subsurface biodegradation, primarily concerned with cleaning contaminated aquifers, have been patented by Raymond and Jhaveri and applied successfully (Raymond, 1974; Raymond *et al.*, 1978; Jhaveri *et al.*, 1981 Jhaveri & Mazzacca, 1983). The technique was varied in the US (Bouwer *et al.*, 1988) and Denmark (James & Sanning, 1989) by addition of an alternative electron acceptor (e.g. nitrate), a microbial inoculum or an additional carbon source, e.g. methane, to enhance the cometabolism of chlorinated hydrocarbons.

Bioreactors have been used for the treatment of contaminated soil or water either in isolation or as an extension of the *in situ* process, e.g. groundwater contaminated with PAH's (Van der Hoek *et al.*, 1989) or benzenoid compounds (James & Sanning, 1989) and former gas works soil (Cuperus, 1988; Van Luin, 1988) .

The potential for successful *in situ* biodegradation of organic compounds strongly depends on the biodegradability of the contaminants and high soil permeability. For optimum biodegradation the soil oxygen content, temperature and rate of saturation are all important. As oxygen appears to be a limiting factor, air-saturated water (8mg O_2/l), O_2-saturated water (40mg O_2/l) or H_2O_2 (100mg O_2/l) can be added. Usually oxygen-saturated water is used, as 200mg/l H_2O_2 is both toxic and is catalytically decomposed by iron salts, though it can be stabilised by phosphate (Staps, 1989a). Biological remediation treatments have the advantages of cheaper costs compared with extraction, and treatment is not confined to site surfaces but among the disadvantages are the difficulties in achieving an even distribution of nutrients and

organisms through the site; possible inability of the microorganisms to degrade compounds strongly adsorbed to aquifer material; *potential* production of by-products more mobile and more toxic than the original contaminant; and soil which can be clogged by excessive bacterial growth, though this is a very rare occurrence (Bouwer *et al.*, 1988; Staps,1989a).

As mentioned on pages 4-9 and 29-34, some of the major organic pollutants of contaminated sites in the US and Western Europe are aromatic compounds. The aerobic metabolism of aromatic compounds by bacteria is dependent on the presence of molecular oxygen for ring hydroxylation to effect ring cleavage.

Hydroxylation of the ring is carried out by a group of enzymes, the oxygenases, of which there are 2 types - the monoxygenases and the dioxygenases. The monoxygenases add a single hydroxyl to the substrate molecule by incorporating one atom of molecular oxygen with the concomitant reduction of the second atom of oxygen to water [e.g., phenol is hydroxylated to catechol (Evans, 1947); *p*-cresol yields 4-methylcatechol (Chapman, 1972)]. The dioxygenases simultaneously add two atoms of molecular oxygen to the substrate molecule forming an ortho dihydric phenol (e.g. dihydroxylation of benzene to catechol (Gibson, 1972).

Under aerobic conditions, the primary pathways usually converge, resulting in key intermediaries in the bacterial degradation of benzenoid compounds; *viz para* or *ortho* di- or tri-hydric phenols prior to ring fission e.g. catechol, protocatechuic acid, gentisic acids and 1,2,4-trihydroxybenzene (from quinol) (Gibson, 1968; Meikle, 1972; Dart & Stretton, 1980) (Fig. 5).

Ring cleavage is achieved by reactions in which both atoms of the O_2 molecule are incorporated into a transient enzyme-substrate intermediate at the same time as the nucleus of the diphenol is cleaved. Two alternative routes exist in bacteria for ring

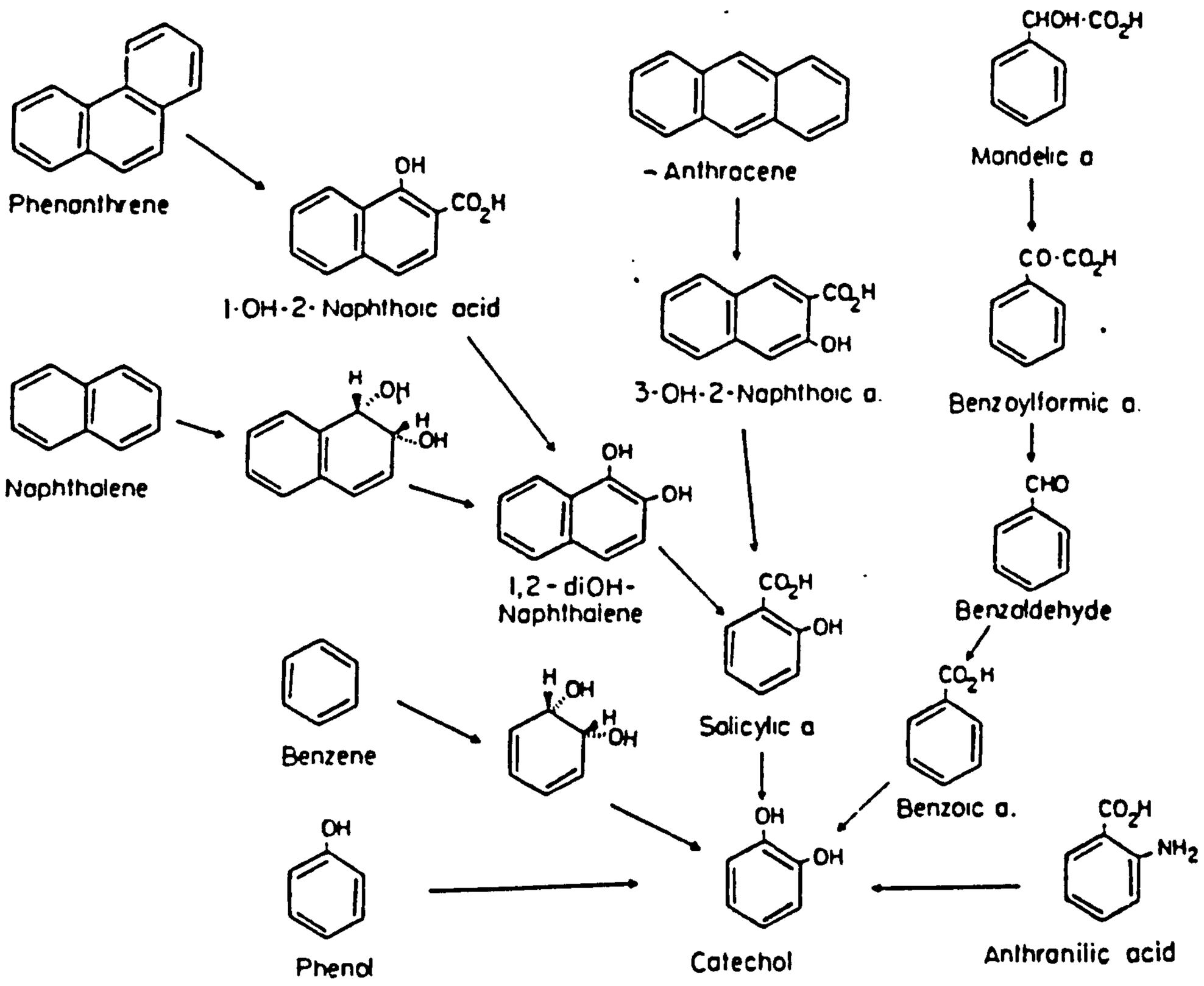


Figure 5. The role of catechol as a central metabolite in the bacterial degradation of benzenoid compounds. (From Chapman, 1972).

cleavage, each initiated by a different kind of dioxygenase attack. Meta fission (cleavage adjacent to one of the two hydroxyls) yields a 2-hydroxymuconate semialdehyde which is finally converted to pyruvate and another carbonyl compound depending upon the ring substituents (Fig. 6). The alternative ortho fission (cleavage between the hydroxyl groups) forms a 6-carbon dicarboxylic acid (e.g. *cis,cis*-muconate) which is eventually converted to succinate and acetyl-CoA by the enzymes of the 3-oxoadipate pathway (Fig. 7).

When the aromatic nucleus is substituted, cresol- and xylenol-utilising micro-organisms initiate degradation either by methyl group oxidation leading to protocatechuic acid or methyl gentisic acid followed by ortho fission, or by ring hydroxylation yielding 3,4-dimethylcatechol followed by meta ring fission (Chapman, 1972). Most Gram-negative bacteria channel alkyl aromatics via the meta cleavage route thus avoiding the retardation or prevention of further metabolism by the formation of dead-end metabolites which methyl intermediate substituents may present (Bayly & Barbour, 1984).

Aromatic compounds can undergo ring cleavage under anaerobic conditions by photosynthetic microorganisms, by respiration carried out in the presence of nitrate and by methogenic bacteria (Evans, 1977) but there is little convincing evidence that hydrocarbons can be anaerobically degraded under any conditions.

Peripheral pathways for organopollutant catabolism are often plasmid-borne. Plasmids are extrachromosomal pieces of DNA coding for their own replication and conjugation, with a varied range of functions which can include the catabolism of novel synthetic compounds and resistance to heavy metals, both of which give their host a potential selective advantage. It is now believed that the plasmid is ubiquitous and that

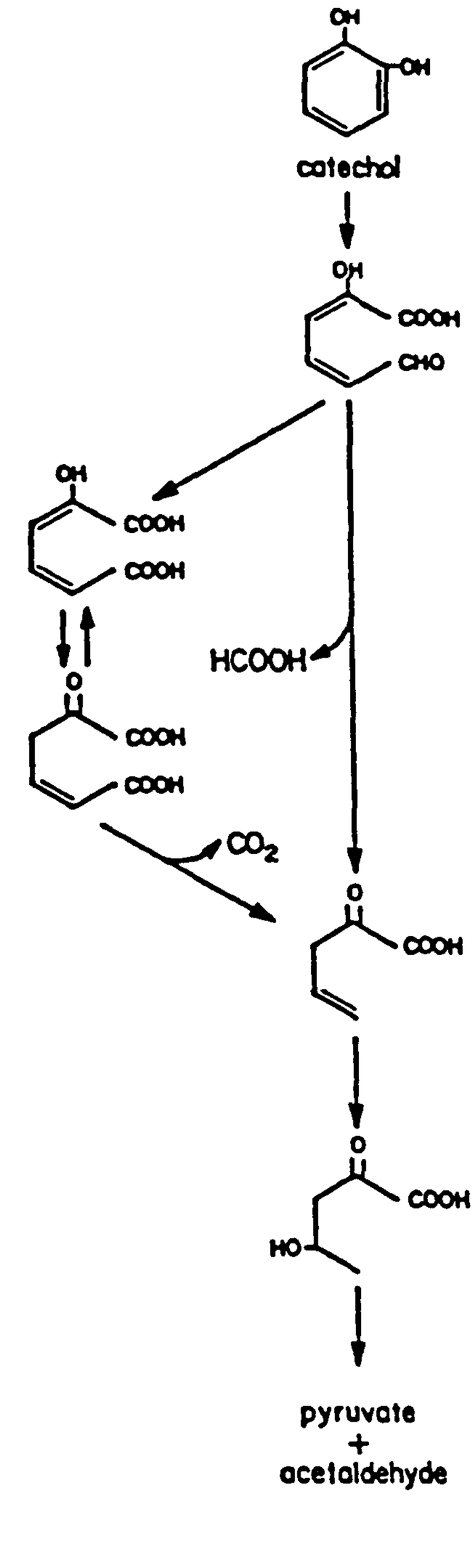


Figure 6. Dissimilation of catechol via the *meta* cleavage pathway (Williams & Murray, 1974).

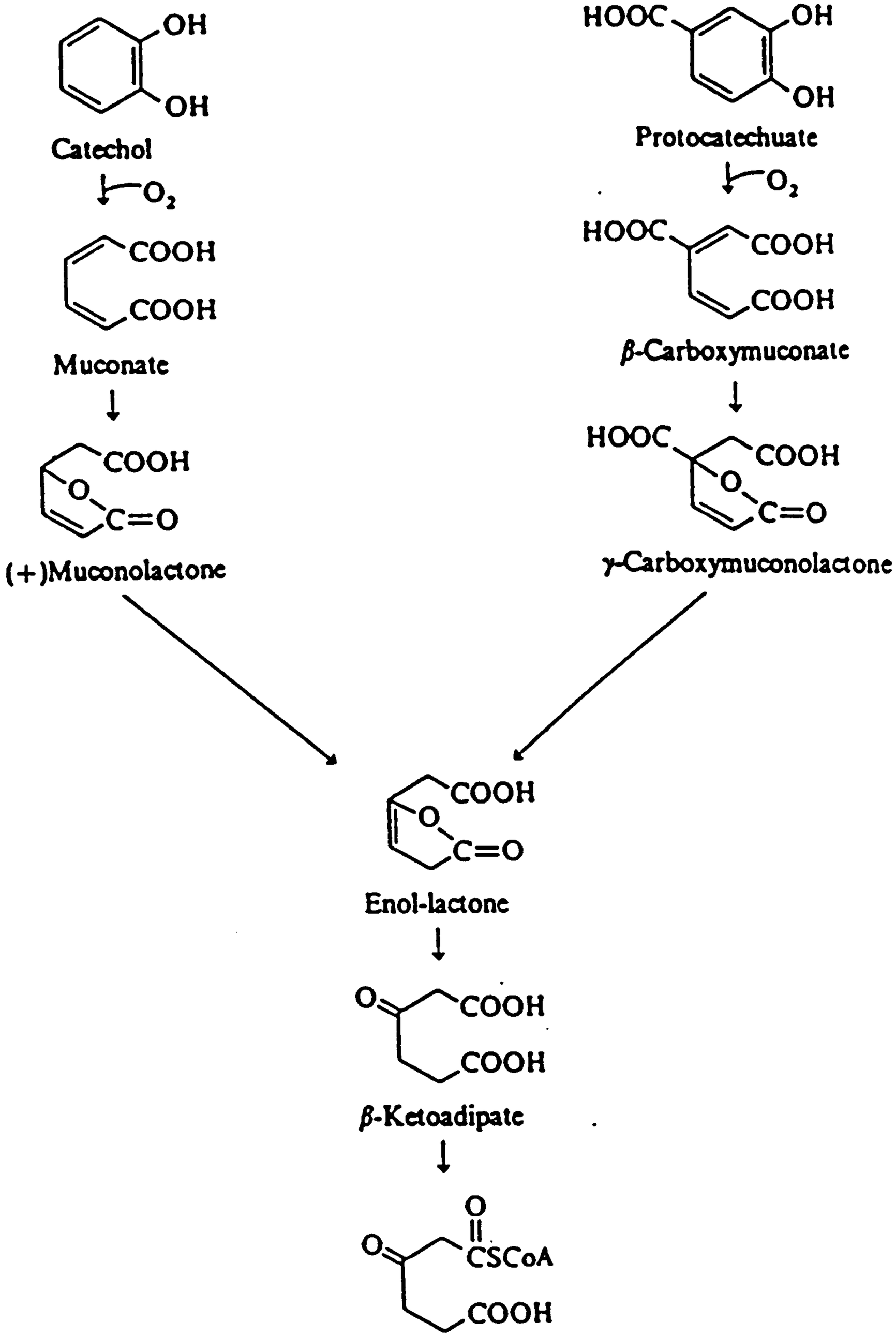


Figure 7. The catechol and protocatechuate branches of the β – ketoadipate pathway (adapted from Clarke & Ornston, 1975).

50-60 % of the wild population of bacteria harbour a plasmid (Williams, 1978). Currently catabolic plasmids are mainly encountered in pseudomonads (Hardman, 1987) which may explain the extensive aromatic catabolic capabilities these organisms possess. The presence of catabolic plasmids in a bacterial community enables the organism to exchange genetic information. Inter- and intra-plasmid recombination may lead to the shuffling of genes on catabolic plasmids, resulting in new combinations of genes which may extend the aromatic catabolic potential and resistance to heavy metals to the host bacterium. Selective pressure acts on mixed microbial communities to encourage the evolution of the new catabolic enzymes. Plasmid frequency increases in populations exposed to chemical stress (e.g. presence of aromatics or inorganic pollutants), depending on the amount and type of chemical (Burton *et al.*, 1982; Day *et al.*, 1988). Hada & Sizemore (1981) found a greater frequency of plasmid-containing organisms in an oil field (35%) than in a similar control site with no oil pollution (23%).

In practice therefore all technologies other than those in which the contaminant is destroyed or rendered harmless, offer a solution of only limited or uncertain duration unless supplementary mechanisms, such as microbial attack, are introduced subsequently. In general, treatment systems based on barrier isolation are likely to lose their effectiveness with time as chemical, biological and physical factors have damaging effects on the barrier. *In situ* microbial methods of treatment are receiving more interest both on environmental and cost grounds and are claimed to offer a saving of 45% over conventional excavation methods for contaminated sites that could be treated in this way (Haines, 1988).

1.6 Availability of contaminants in soil to plants

Soil is made up of organic and inorganic constituents. The organic phase or humus consists of a large number of compounds which are formed by decomposition of plant and animal residues and from the synthetic activities of microorganisms. The chemical composition of the humus depends largely upon the type of organic residues and the environment under which the decomposition is occurring. Lignin and humic compounds are the main constituents (Foth, 1990). Phenolic compounds may account for up to 30% of the weight of the humic polymer (Bollag & Loll, 1983).

Humus has a large surface area and humic substances contain large numbers of negatively-charged reactive (usually carboxyl) groups which play an important role in the adsorption of cations, bonding of water and cohesion of the soil particles i.e. clay and other humic and fulvic acids (De Hann & Van Riemsdijk, 1986). Xenobiotics structurally similar to humus constituents, e.g., 1-naphthol, may be incorporated into soil humus as part of its synthesis by binding to humic substances (Bollag & Loll, 1983). Evidence suggests this binding may be temporary as microorganisms (Mathur & Morley, 1975) can release them by using the organic ligands as carbon and energy sources.

The sorption of organic compounds to soil organic matter is influenced by a variety of compound properties. Volatility, charge, polarity and molecular structure are all factors but the most important one is thought to be the water-solubility of the organic compound (Pancorbo & Varney, 1986), though there is evidence that water-immiscible compounds like alkanes, form complexes with humus by reacting with non-polar components of soil organic matter. These reactions could influence the movement of xenobiotics in soil and water (Bollag & Loll, 1983).

The mineral fraction of the soil originates from the disintegration and decomposition of the parent rock and is composed of particles of a variety of sizes. Due

to its large surface area, clay is the most important particle in terms of reactivity, such reactivity being a function of the external surface area and surface charge density (Alexander, 1965; De Hann & Van Riemsdijk, 1986). Clay particles are negatively charged so to balance the negative charge, clay particles tend to adsorb counterions (cations) K^+, Zn^{2+}, Cd^{2+} which are differentially adsorbed depending on their size, charge, hydration and the type of mineral involved. Ions of higher valency remain associated with the clay surface in the presence of lower valency ions and will displace lower valency ions from the clay surface. When ions have the same valency, the replacing power tends to increase with the size of ion. Cation exchange capacity (CEC) is a function of the amount and types of organic matter and clay minerals in the soil. The organic portion of the soil contributes to its cation exchange capacity as removal of this fraction (only 3-5% of total soil mass) reduces CEC by between 20-50% (Alexander, 1965; Paul & Ladd, 1981).

The negative charges of the clay minerals and humic substances make adsorption and exchange one of the most important features in soil, since it is this property that determines the capacity of a soil to store the majority of the essential plant nutrients, metal ions and organics (Sposito, 1986). Organic compounds, e.g. phenolics, can be stabilised by sorption onto clays and humic materials both chemically and by microbial processes. Soils with a high clay and organic matter content tend to attenuate the toxic effects of phenol due to formation of H bonds between the soil colloids and the negatively charged OH group of phenols (Cheng *et al.*, 1983; Isaccson, 1985).

The reactive surfaces of metal (hydr)oxides are capable of adsorbing both cations and anions through the formation of surface complexes between the reactive surface oxygen groups of the metal (hydr)oxides and the adsorbing species (Van

Riemsdijk *et al.*, 1990). The availability to plants of the anions - phosphate, nitrate and sulphate - is related to anion exchange as well as mineralisation from organic matter (Foth, 1990).

The ability of soil organic matter to form stable combinations with metal ions has been well established. The high molecular weight, largely insoluble humic and fulvic substances and a myriad of lower molecular weight individual biochemical substances, including organic acids, amino acids and phenolics, can bind metal ions by the formation of organometal complexes of different stability and solubility (Stevenson & Ardakani, 1972; Piccolo, 1989). Metal ions in soil may exist in a number of forms:- a) metal ions can react with dissolved inorganic or organic ligands to form a soluble complex ion, b) metal ions can react with surface ligands on clay or organic matter to form complex ions associated with the solid phase c) metals ions can be adsorbed by microorganisms or plant roots and may/may not be transported into living material (Fig. 8). Clay particles may also fix appreciable quantities of metals by precipitation (forming an insoluble salt), by the physical entrapment of metals in clay lattices and by exchange adsorption on the surface (Tyler, 1981). The stability of the complex ion increases with the electro-negativity of the metal. As the complex ion bears the net charge, it attracts other ions forming chelated groups (Stevenson & Ardakani, 1972). Although rankings change for different soils, the stability of complexes formed by a ligand and a series of divalent metal ions generally decreases in the order Pb > Cu > Ni > Co > Zn > Cd > Fe > Mn > Mg (Irving & Williams, 1948). Although this order may vary according to the nature of the ligands, pH and interactions with other ions, it predicts that when these metals are added to soils with organic residues, the relative increase in availability of (say) Mg should be greater than for Pb.

Availability of metals to plants is dependent on: pH, redox potential, mineral

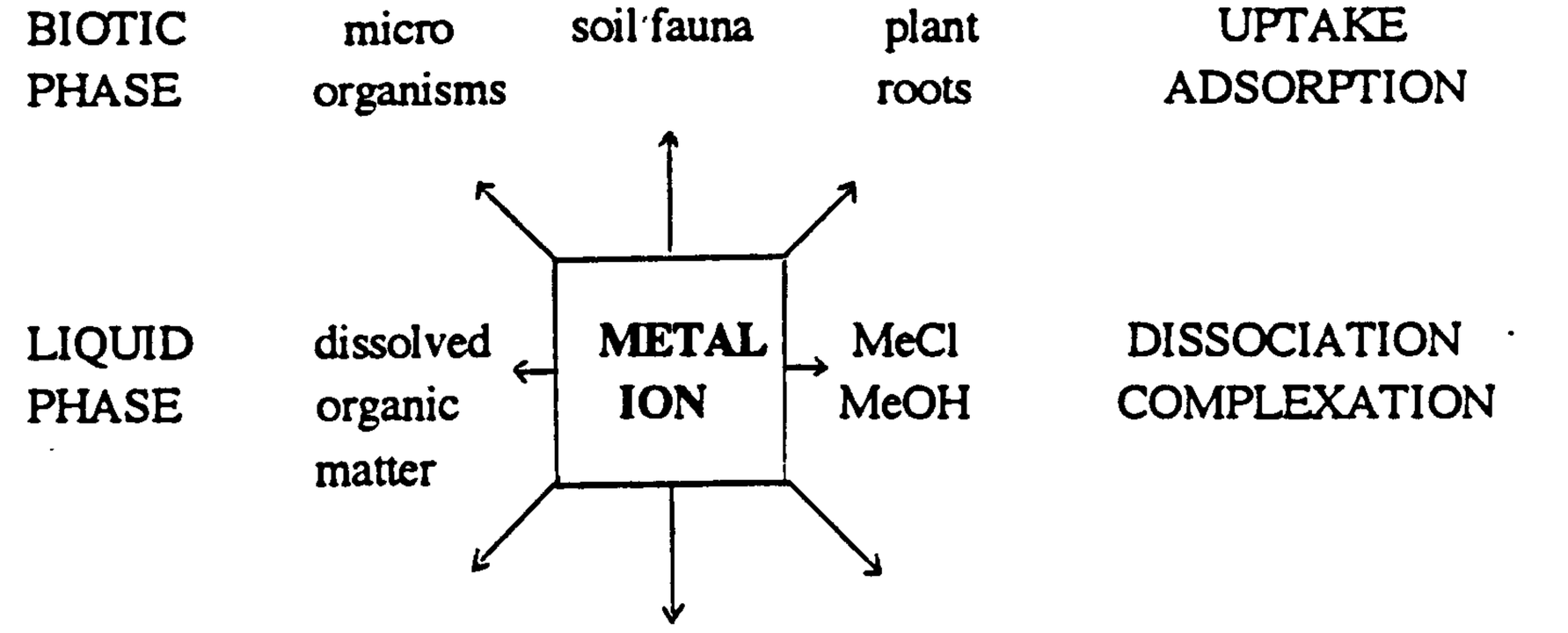


Figure 8. Schematic representation of the various interactions of a dissolved metal ion in a soil environment (from Riemsdijk *et al.*, 1990).

composition, soil temperature, moisture content, soil CEC and the chemical form of the metal, and bioassay is a more sensitive probe to extractants which have their limitations and may thus represent a more valid measurement of metal availability to plants. This is especially true where more than one metal is present in the soil and their effect is synergistic (Lehn & Bopp, 1987; Påhlsson, 1989; Kovacs & Podani, 1986). Plants have been used to assess available metals in soil for many years; e.g. Bould *et al.*, (1960) used leaf analysis as a guide to fruit crop nutrition. More recently, Davis *et al.*, (1978) has made use of barley harvested at 5 leaf stage in determining critical plant tissue concentration. Lehn & Bopp (1987) have presented evidence to suggest the use of seedlings as indicators for the amount of heavy metals in mature plants and Whitton *et al.*, (1981) used plants to monitor heavy metals in rivers. Davis (1984) has suggested that extractants were best used not to assess availability directly but to identify the major forms of metal in the soil by sequential extraction techniques, i.e. exchangeable, sorbed, organic carbonate and sulphide forms.

1.7 Plant growth in metal-contaminated soil

A few heavy metals, including Fe, Cu and Zn, are essential nutrients for enzymatic function in plants and microorganisms but the requirement for such metals is small and excess amounts are frequently inhibitory or toxic to cellular activities, restricting their growth, diminishing their productivity or biomass (Bååth, 1989; Crowder, 1991). Contaminants follow the pathways of uptake used by nutrients. Heavy metals generally inhibit physiological processes eg. photosynthesis, phloem translocation and transpiration; respiration is less sensitive. A further response is the increase in activity observed for several enzymes eg. peroxidase, glucose-6-phosphate dehydrogenase (Van Assche *et al*, 1988). When plants assimilate phytotoxic amounts

of metals such as Zn, Cd and Cu, growth becomes inhibited and biomass production decreases (Lepp, 1981). Stunted growth, leaf epinasty and chlorosis are visible symptoms of strong metal phytotoxicity. At lower levels of soil pollution, however, these visible symptoms may become less evident, but reduction of plant quality and biomass production can persist (Van Assche & Clijsters, 1990).

The toxicity of metals is due primarily to their effect on metabolic processes in the plant (enzymes), through 1) inhibition by the masking of active groups; e.g. much higher affinity of Cd ions for thiol groupings (SH) in enzymes and other proteins (Bould *et al.*, 1960; Påhlsson, 1989; Tyler *et al.*, 1989; Van Assche & Clijsters, 1990); 2) inactivation by affecting enzyme conformation; 3) protein denaturation, or 4) by forming precipitates or chelates with essential metabolites. Alternatively, metals can act competitively for enzyme substrate sites, e.g. Zn competes with Fe decreasing chlorophyll content in chlorophyll biosynthesis (Carlson *et al*, 1975; Clijsters & Van Assche, 1985).

In plants, seed germination appears to be insensitive to Cu and Zn. Roots act as a barrier to metal uptake by the shoots by accumulating excess metals (Hardiman *et al.*, 1984; Bakhsh *et al.*, 1990) to the extent that the health of roots, in particular root length, is widely used to assess the tolerance of plants to metals. Cell membranes in roots are generally considered primary sites of metal injury (Påhlsson, 1989; Barcelo & Poschenrieder, 1990) where increased permeability leads to a leaky plasmalemma. These early events lead to a large range of secondary effects such as deficiency of essential nutrients, inhibition of photosynthesis and alterations of water relations which further reduce shoot and root growth (Barcelo & Poschenrieder, 1990). As a result leaves become chlorotic and plants become water stressed and stunted (Tyler *et al.*, 1989; Förstner, 1988). Generally most species are sensitive to metals in the range (μg/g dry wt.): 200-300 Zn (Påhlsson, 1989); 15-20 Cu (Påhlsson, 1989); 3-10 Cd (Connell

& Miller, 1976) and 100-200 Pb (Påhlsson, 1989).

While Zn and Cu may be translocated to the aerial parts of plants, where they display symptoms of phytotoxicity, though still relatively harmless to human or animal health, Cd and Pb are poorly translocated. Lead, in particular, is accumulated in the roots and translocation to aerial shoots is limited (John & Van Laerhoven, 1972). Cadmium is also bound to cell walls in roots and only limited translocation to shoots occurs.

Some authors (Davis, 1984; Chang *et al*, 1987) have found a positive relationship between soil and plant Cd concentration though the relationship appears to differ with soil Cd, concentration, soil type and crop species. Extractable and soil solution concentration generally depend on a) the total metal concentration in soil b) the soil pH and c) the soil`s CEC (Sanders & Adams, 1987).

1.7.1 Mechanisms for resistance in plants

The uptake of an excess of heavy metals by plants can be self-regulated in several ways such as, metal binding to the cell wall, compartmentalisation in vacuoles, detoxification through chelation either internally or externally (Van Assche & Clijsters, 1990) or though the evolution of tolerant enzymes (Taylor, 1987). Recently the presence of phytochelatins (PC) has been discovered in higher plants. Phytochelatins are functional analogues to metallothioneins, sulphur-rich proteins which detoxify heavy metals in vertebrates and some fungi. They are low molecular weight peptides, with a high cysteine content, which are specifically induced in plants occupying heavy metal-containing ecosystems (Robinson & Jackson, 1986; Grill *et al.*, 1988; Jackson *et al.*, 1987). Tolerance is both species-specific and metal-specific. Cadmium appears to be the strongest inducer, while zinc requires very high levels for induction, maybe

reflecting their differing levels of inherent toxicity to the plant (Steffens, 1990). Root tissue has been found to contain much higher concentrations of heavy metals and phytochelatins than leaf tissue suggesting that metals are immobilised to a greater extent at the site of metal uptake (Grill *et al.*, 1988). The presence of phytochelatins in all the major plant classes, families and orders has been confirmed by Gekeler *et al.*, (1989).

1.8 Effect of metals on microorganisms

Heavy metals present in high concentrations are toxic to all organisms. Microorganisms are no exception and exposure to heavy metals affects their growth and survival. Generally soils that have received high levels of metals (even 20 years ago) show reduced microbial abundance and biomass together with lower microbial activities than soils which have not (Brookes *et al*, 1984). Decreased soil enzymic activity and respiration, leading eventually to decreased organic litter decomposition (Doelman & Hååristra, 1979a) are common features of heavy metal pollution of soils indicating either toxic effects on soil microbiota and extracellular soil enzymes or that metals may render low molecular weight organic compounds (e.g. citrate) resistant to bacterial attack (Brynhildsen & Rosswall, 1989). Soil respiration appears to be a sensitive measurement with which to detect heavy metal pollution (Doelman & Hååristra, 1979; Bååth, 1989; Tyler, 1981).

A reduction in the number and diversity of microorganisms because of the presence of heavy metals, has been detected in numerous investigations along with the development of tolerant or resistant strains. The percentage of sensitive microorganisms in the soil increases with the toxicity of metals (i.e. Pb < Zn < Cu < Cd), their concentration and the total number of metals present (Olson & Thornton, 1981). The high abundance of metal-tolerant fungi in polluted soils is thought to be due to a shift in

species composition to metal-tolerant species, while in bacteria where metal-resistance is usually plasmid encoded, tolerance is more likely to be due to gene transfer via plasmids encoding metal resistance (Mergeay, 1991). While plasmids conferring multiple resistance to heavy metals are found in both Gram-negative and Gram-positive bacteria, resistance to high concentrations (1-10mM) was only found in Gram-negative bacteria which were mainly isolated from soils with high concentrations of heavy metals and often associated with very large plasmids carrying multiple resistance genes (Mergeay, 1991). Nitrifying bacteria, the nitrogen-fixing *Azotobacter* and mycorrhizal fungi, especially those forming VA mycorrhiza, have all been reported to be particularly sensitive to high levels of heavy metals (Tyler *et al.*, 1989).

Biochemical compounds with chelating characteristics e.g. acidic polysaccharides and amino acids, are continuously being produced by microorganisms in the soil. These constituents normally have only a transitory existence in the soil solution as they are continually being turned over by microorganisms. Appreciable quantities may be synthesised during periods of intense microbial activity, however, resulting in high concentrations in localised zones where biological activity is high, e.g. in the rhizosphere and near decomposing plant residues. Such chelating compounds, produced by microorganisms or excreted by plant roots, influence the metals (e.g. Mo, Fe, Mg, Ca, K) held in the soil solution as free ions and soluble metal chelates, and act as agents for the movement of micronutrients to roots, to satisfy the specific requirements of metal-enzymes and/or structural components of the cell (Stevenson & Ardakani, 1972). In particular, plants with mycorrhizal infections have been shown to display enhanced metal uptake to plant roots (Dehn & Schuepp, 1989), where the metals are bound to metallothioneins located at the cell wall, and not translocated to the plant shoot. Although most fungi use metallothioneins for sequestering heavy metals, a

fungus, (*Candida glabrata*) has for the first time been found to expresses both metallothioneins and phytochelatins (Mehra, 1988).

Objectives

The increasing pressure for land, particularly in the inner cities, has made the redevelopment of former contaminated industrial sites an attractive proposition. Sediment, dredged from the River Tyne at Dunston basin, Gateshead, Tyne & Wear, intended for land contouring at the Gateshead Garden Festival 1990 and soil from the now derelict Derwenthaugh (DWH) coke works site at Blaydon on the outskirts of Newcastle, in which excessive contamination levels have prevented redevelopment were both examined as candidates for remediation.

The overall objective of this project was an examination of methods for the remediation of two quite different contaminated soils. Soil on the DWH site was heavily contaminated with hydrocarbons, phenols and inorganic contaminants. The sediments at Dunston Coal Staiths were thought to be contaminated by run-off from the from the DWH and Redheugh coal carbonisation sites but contained heavy metal contaminants. Amelioration of the toxic effects of heavy metals in weathered sediment dredged from the River Tyne was attempted by the addition of lime, fertiliser and organic matter to field plots of the dredgings. The biodegradation of organic pollutants in the soil contaminated with coal carbonisation waste, was examined under optimized environmental conditions favourable for aromatic degradation with microbes, previously isolated and enriched on cresol and thiocyanate, inoculated into the soil.

The specific objectives were to:-

1) Identify contaminants in dredged material and DWH soil with particular reference to the characterisation of phenols.

2) To establish experimental plots to investigate the effect of metal contamination on plant growth in River Tyne dredgings and to moderate its phytotoxicity.

3) To examine means to remediate the phenolic contamination present in the Derwenthaugh soil.

MATERIALS AND METHODS

2.1 Chemicals

Chemicals and solvents (analytical or HPLC reagent grade) used in this work were purchased from BDH Ltd. (Poole, Dorset, UK) unless otherwise stated.

2.2 Site Identification

Ordinance survey maps, local and technical literature were used to identify former coal carbonisation sites and parts of the site where soil contamination might be present. Site reconnaissance was undertaken to observe the present state of the site and clues indicating the location of previous site buildings. The presence of surface waste deposits, odours and presence or absence of vegetation were also noted (Lord, 1981; Department of the Environment, 1983).

2.3 Soils

Dredgings were obtained from Dunston Coal Staiths basin on the River Tyne, with the permission of Gateshead Metropolitan Borough Council (Fig. 9). The material used had been dredged April/May 1987 from Dunston basin and allowed to partially weather in mounds 2.5m deep (Photograph 1). One tonne of this material was removed by JCB from a central part of the site and transported to Moorbank Experimental Gardens of the University of Newcastle, Claremont Road, Newcastle-upon-Tyne. Soils used in degradation studies and as microbial inocula were obtained from disused coke works sites at Dunston on the River Tyne and at Derwenthaugh, nr. Blaydon, Tyne & Wear; control, pesticide-free, arable soil was collected from the University agricultural field station (Close House, Heddon-on-the-Wall, Northumberland).

At the Derwenthaugh site, an area (5m x 5m) with very dark oily surface waste deposits, a pungent odour and devoid of any form of vegetation was selected for

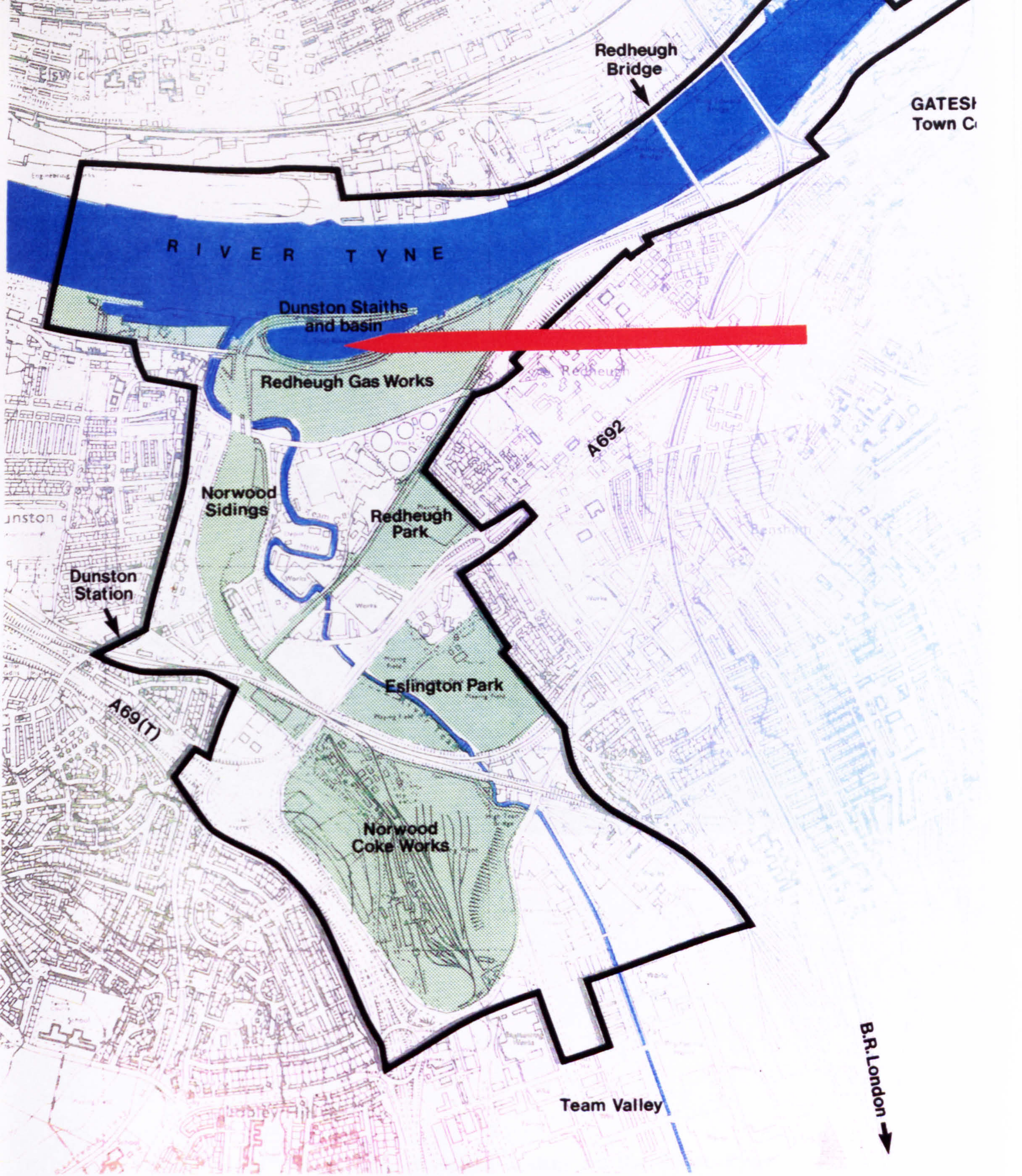


Figure 9. Plan of National Garden Festival Site at Gateshead 1990.

Arrow indicates site of sampling.

Photograph 1. **Dunston Staiths on the River Tyne, Newcastle, after dredging operation - March 1988.**

sampling (Photograph 2). A stainless steel spade was used to sample the top 30cm. Three samples (2kg each) were collected in large labelled polythene bags (Mitchell, 1960). Soil was also sampled at different depths of 15, 21 and 30cm., to determine the extent of contamination.

The surface samples were spread onto a clean surface, bulked and thoroughly mixed to provide one sample for analysis. Subsampling was obtained by quartering the whole sample until the required weight was obtained. Any material greater than 2cm (max diam.), which was not pertinent to analysis e.g., stones, bricks were removed. The other material was broken down by wooden roller until it was less than 2cm diam. (Lord, 1981; Mitchell, 1960). The soil was dried at room temperature for 5 days, and sieved to 2mm, (Wilson & Stevens, 1981) prior to analysis. All samples were stored at 4°C in the dark in sealed glass jars (Environmental & Pollution Standards Committee, 1983). When sampling for microbial analysis, mixed composite samples were taken using sterile implements and containers. Care was taken not to expose samples to undue heat or drying. They were stored at 4°C and analysed within 18h (Williams & Gray, 1973).

2.4 Soil Analysis

All glassware was cleaned in hydrochloric acid (1M), rinsed in double distilled water, oven-dried and solvent-rinsed if appropriate, before use. Likewise alumina used for thin layer chromatography was Soxhlet extracted with dichloromethane, then dried at 30°C before activation and stored in an oven at 120°C, prior to use. Solvents used for gc-ms work were of reagent grade and were redistilled in an Oldershaw column, except for carbon tetrachloride where Fisons HPLC grade was used. The purity of each batch of solvent was tested prior to use by evaporation of 5ml under a stream of dry nitrogen followed by analysis of a 1μl aliquot by gas chromatography. The term "light petroleum" refers to the fraction with a boiling range between 40°C and 60°C.

Photograph 2. **Disused coke works site at Derwenthaugh, Blaydon, nr. Newcastle.** Soil for biodegradation experiments was taken from the area (5m x 5m) in the foreground.

Infra-red (i.r.) spectra were run on a Nicolet 20 PCIR spectrophotometer (Nicolet Instruments, Budbrooke Rd., Warwick, England). Samples of oven-dried soils were run as potassium bromide-admixed discs (ratio of sample to KBr = 1:300). A second spectrum was then run with a dichloromethane extract of the same dredged material.

One of the most useful methods for measuring *total hydrocarbon content* is infrared spectrometry, using carbon tetrachloride as an extractant, as it produces little or no spectral interference in the i.r. spectral region of interest. I.r. analysis was used to measure residual hydrocarbon in the Zurich test, (Section 2.6.2) by measuring maximum absorption of CH_3-CH_2 band at 2930 cm^{-1}. Extractions of samples were carried out using a Soxhlet apparatus with carbon tetrachloride (Fig. 10). The soil samples were placed in pre-weighed cellulose extraction thimbles (Whatman, double thickness) and extracted for 12h with spectroscopically pure carbon tetrachloride. The extract was adjusted to 100ml with carbon tetrachloride. Aliquots were then diluted with carbon tetrachloride sufficiently to produce a marked deflection in the i.r. spectrum between 3800cm^{-1} and 2500cm^{-1} on a Perkin-Elmer infra-red spectrophotometer model 297 (Fig. 11). The infra-red absorbance of the hydrocarbon peak at 2940cm^{-1} was measured on a sample of the diluted extract and calculated as follows:

A baseline was constructed between the transmittance maximum at about 3000cm^{-1} and 2840cm^{-1}. A vertical line was constructed from the peak maximum at about 2940cm^{-1} to intersect the baseline. The transmittance values at the peak maximum (I) and the point of intersection between the vertical line and the baseline (I^o) were measured. The value of absorbance (A) was calculated where

$$A = \log_{10} \frac{I_o}{I}$$

The hydrocarbon content of the extract was obtained by reference to a calibration graph prepared from standard solutions of hexadecane (10-40mg/100ml).

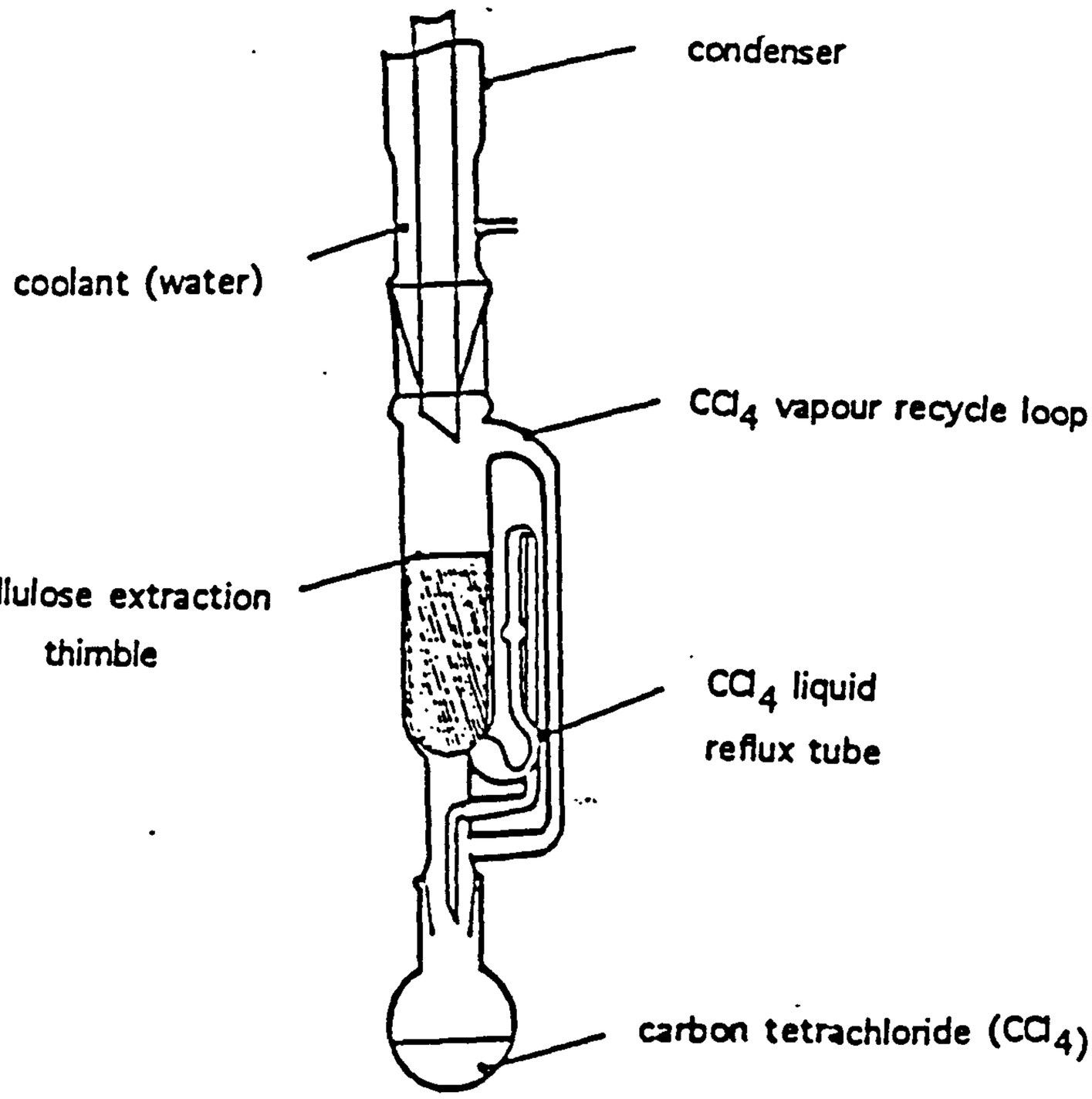


Figure 10. Soxhlet tube assembly used for total hydrocarbon content analysis.

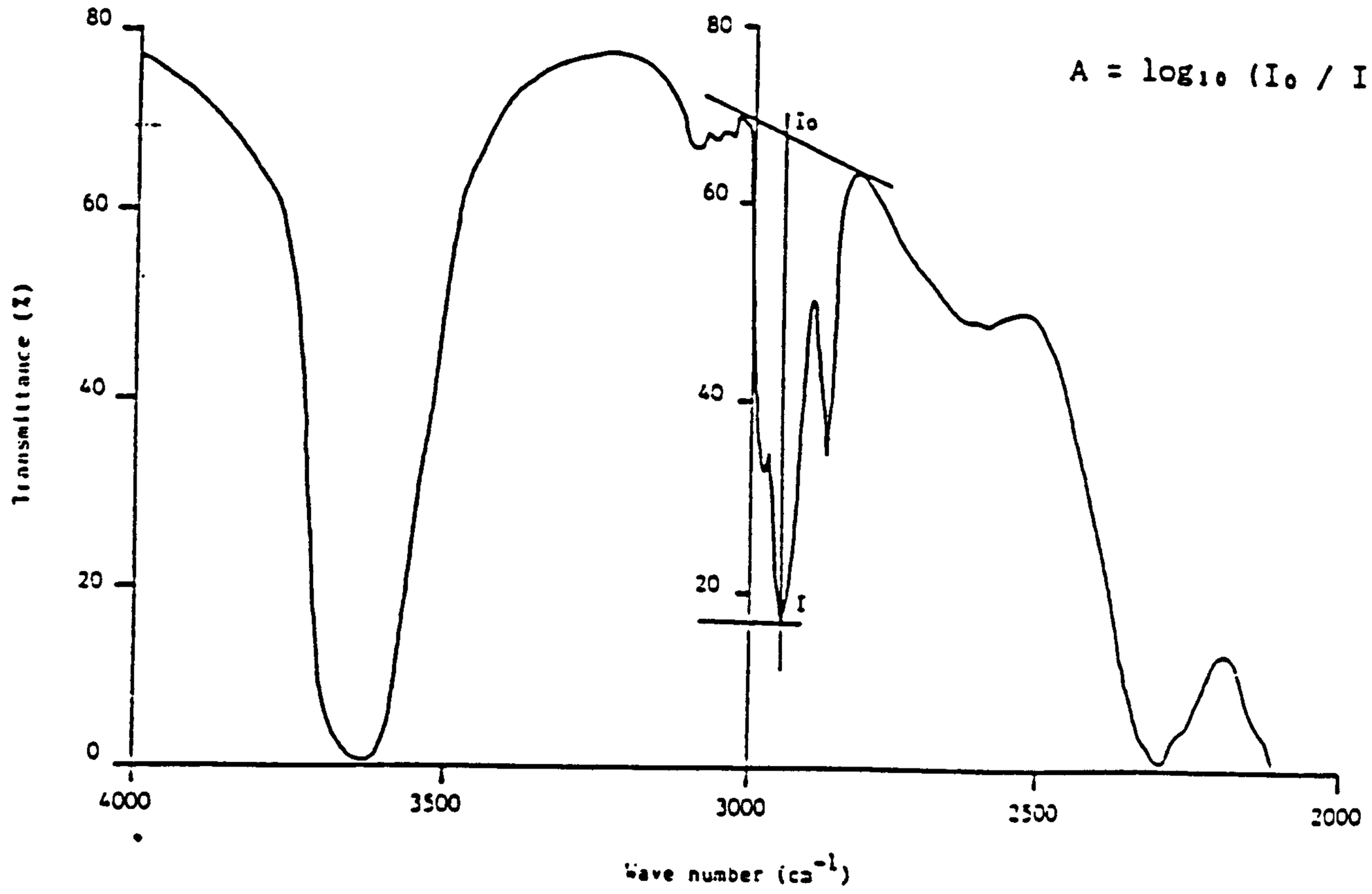


Figure 11. Infra-red spectrum of a diluted spectrum of a spectroscopically pure CCl_4 extraction of Derwenthaugh soil. The figure shows how the absorbance of extracts was calculated from the transmittance readings at 2940cm^{-1}

$$A = \log_{10} (I_o/I)$$

pH values of the soils were measured by shaking 20g samples with 50ml distilled water for 10min. After filtration, the pH was measured at 20°C using a standard laboratory pH meter (Jenway 3020).

Organic carbon content was determined by quantitative ashing of the soil, (Allen *et al.*, 1974). The results from quantitative ashing of soils are usually measured as loss on ignition and, as such, are a rough indication of the amount of organic matter present in the soil. Loss on ignition is not a true measure of organic matter because at the temperature of ashing some bound water is lost from the clay minerals and is included in the overall loss. Oven-dried soil (1g, 100°C) in a weighed, dry crucible was placed in a muffle furnace, the temperature raised slowly to 450°C and allowed to remain at that temperature for 4h. After cooling, the crucible was transferred to a desiccator and subsequently weighed. Percentage loss on ignition from weight loss during combustion was calculated:-

$$\text{Loss on ignition (\%)} = \frac{\text{wt. loss (g) x 100}}{\text{oven-dry wt. (g)}}$$

Water content (air-dry moisture) was determined using the method by Allen *et al.*, (1974). Sieved (2mm), air-dry soil (1g) was placed in a preweighed crucible and dried in an air circulation oven at 70°C for 8h or until a constant weight as recorded. The crucibles were cooled in a desiccator and reweighed.

$$\text{Dry matter (\%)} = \frac{\text{oven-dry wt. (g) x 100}}{\text{initial sample wt (g)}}$$

Ammonium-N was determined colorimetrically by a modification of the indo-phenol blue method. Three controls (blanks) and three replicates of air-dry Tyne dredged material (with known moisture level) (3g) were shaken in 250ml flasks for 1h at 25°C with 50ml of K_2SO_4 solution (0.5M). The solution was filtered (Whatman

No.42) and kept at 5°C until analysed using a Technicon 2 autoanalyser (Technicon Ltd., Dublin, Ireland).

The standard $BaCl_2$ turbidimetric method [American Public Health Association (A.P.H.A.), 1983] was used to determine *sulphate*. Triplicate samples (2.5g) were weighed into 100ml flasks, 100ml HCl (5%) was added and samples boiled gently for 5min. The samples were cooled and filtered (Whatman No.1) after which 5ml of conditioning reagent (50ml glycerol; 30ml concentrated HCl; 300ml distilled water; 100ml 95% ethyl or isopropyl alcohol; 75g NaCl) was stirred into each sample. $BaCl_2$ crystals (1.5g approx.) were added and the resultant solution stirred for exactly 1min at which point the OD_{420} was read (Beckman DU 7 spectrophotometer). The reading was then repeated at 30s intervals for 4min and the sulphate determined from a similarly prepared calibration curve (0-40mg/l). Corrections for colour and turbidity in the sample were made by running blanks from which $BaCl_2$ was omitted.

Sulphide was determined iodometrically (Wilson & Stevens, 1981). An oven-dried soil sample (2g) [in triplicate] was weighed into a 50ml round-bottomed flask fitted with a reflux condenser and separating funnel and side-arms permitting a flow of nitrogen to the flask bottom and take off at the condenser top. After purging the apparatus with nitrogen, 25ml of sulphuric acid (10%) was added via the separating funnel, the mixture boiled for 5min and the evolved hydrogen sulphide absorbed in 25ml water containing 2ml of 2M zinc acetate solution. While boiling, nitrogen was bubbled through the soil-acid mixture at a flow rate of 400ml/min into the zinc acetate solution and continued for a further 7min after cessation of heating. Sulphide in the zinc acetate solution was determined by a standard iodometric method (A.P.H.A., 1983). Approximately 20ml iodine solution [(0.025N) I_2 in KI, standardised against $Na_2S_2O_3$ (0.025N) using starch solution as an indicator], was measured into a 500ml flask from a burette and 2ml HCl (6N) added. The Zn sulphide-containing $ZnCl_2$ sample (200ml) was pipetted into the flask, discharging the contents beneath the surface. If the iodine

colour disappeared, more iodine solution was added so that the colour remained. The contents of the flask were then back-titrated with $Na_2S_2O_3$ solution (0.025N), with the addition of a few drops of starch solution as the endpoint was approached and continued until the blue colour disappeared. Sulphide was calculated using the following equation where 1ml 0.025N iodine solution reacts with 0.4mg sulphide:-

$$mg\ S/l = \frac{[(A \times B) - (C \times D)] \times 1600}{ml\ sample}$$

where A = ml of iodine solution

B = normality of the iodine solution

C = ml of $Na_2S_2O_3$ solution for back titration

and D = normality of the $Na_2S_2O_3$ solution

Phenol, thiocyanate and cyanide (Wilson & Stevens, 1981) were determined on the filtered leachate obtained by shaking 10g soil samples with 100ml of NaOH (1M) for 24h at 25°C.

By using a mild alkaline extractant phenolics reversibly adsorbed to soil particles were recovered as their phenates (Whitehead *et al.*, 1981). For the determination of *phenols* the well-established aminoantipyrine colourimetric method was chosen initially as suggested by Wilson & Stevens (1981).

Steam distillation of the volatile phenols from a solution acidified with phosphoric acid was required as the condensation reaction also works with aliphatic amines. The filtered leachate (in triplicate) was acidified to pH 4 with H_3PO_4, steam distilled and the condensate made up to 100ml with distilled water. To the sample 2.5ml of NH_4OH (0.5M) was added and the pH adjusted to 7.9 (±0.1) with phosphate buffer (104.5g K_2HPO_4 and 72.3g KH_2PO_4 per litre double distilled water). 4-Aminoantipyrine solution (1ml of a 2g/100ml solution in distilled water) was added

while stirring, followed by 1ml of $K_3Fe(CN)_6$ solution (8g/100ml distilled water). After 15min, the absorbance was read at 500nm and the concentration of phenol determined from a similarly prepared calibration curve (0-0.5mg phenol/l).

Folin and Ciocalteau`s colorimetric test for phenols was also used where total phenols were to be measured (Box, 1983). A solution of 0.15ml $NaCO_3$ (200g/l) was added to 1ml of sample supernatant and mixed thoroughly. After the addition of 0.05ml of Folin and Ciocalteau`s reagent and subsequent mixing, samples were left at room temperature (20°C) for 60min prior to absorbance measurement at 750nm. A calibration curve was prepared from *p*-cresol (0-1mg/l).

Thiocyanate was determined by measurement of the complex formed with ferric iron in acid solution (A.P.H.A., 1983). The alkaline extract used for phenol distillation (in triplicate) was distilled (moderate boiling for 30min) to remove organic interferences and then adjusted to pH 5 to 7 with (conc.) HNO_3. The samples were transferred to a 50ml volumetric flask and 5ml of $Fe(NO_3)_3$ solution [50g $Fe(NO_3)_3$ in 500ml distilled water, with 25ml of conc. HNO_3, diluted to a litre] was added. The pH was again adjusted to pH 1-2 (if necessary) with HNO_3, diluted to volume and absorbance was read at 480nm, using distilled water taken through the procedure as a blank. Thiocyanate was determined by reference to a similarly prepared calibration curve using KSCN standards (50-500μg/l).

Attempts were made to analyse KSCN by HPLC at 200 and 215nm with various ratios of acetonitrile:water. Although a satisfactory linear calibration curve was obtained with KSCN alone in water, in a 60:40 acetonitrile:water solvent system at 215nm (ret. time 1.68min), satisfactory results could not be obtained with minimal salt media + KSCN because of interference from the mineral salts.

Cyanide was measured semi-quantitatively using the spot test (A.P.H.A., 1983) to establish if more than 50μg/l free cyanide was present in soil samples. The spot test is based on the method in which Chloramine T is used to convert cyanide to

cyanogen chloride (CNCl), which is then reacted with pyridine and barbituric acid giving a red coloured complex. The alkaline extract of soil was heated to boiling and distilled, the distillates (20ml) being collected in a beaker of alkali (20ml NaOH [1M]) then neutralised with HCl. One drop of chloramine T solution [1g/100ml distilled water] was added to 3 drops of sample in a stoppered volumetric, and mixed well. One drop of pyridine-barbituric acid solution [15g barbituric acid; 75ml pyridine; 15ml conc. HCl; made up to 250ml distilled water] was added and the solution mixed. After 1min the colour of the sample spots were noted. A pink to red colour indicated the presence of more than 50μg/l cyanide. Standards of 50μg/l potassium cyanide were used for colour comparison.

To analyse *metals in soil*, cadmium, copper, lead, nickel and zinc extractable in soil, were determined by EDTA extraction (Ministry of Agriculture, Fisheries and Food, 1986). A soil sample (10g) was placed in a 100ml flask and 50ml of ammonium ethylenediaminetetra-acetate (EDTA) 0.5M, at pH 7 was added. The solution was shaken for 1h at 20°C and filtered (Whatman No. 40). The filtrate was retained for metal determination. Three blanks were also run. Standard solutions of each metal 0-2μg/ml cadmium and copper; 0-5μg/ml lead; 0-7μg/ml zinc and nickel were prepared in 0.05M ammonium EDTA (Spectrosol standard solutions, [1000ppm], BDH). The concentration of the elements in the extracts was determined by atomic absorption spectroscopy (Video 12 aa/ae spectrophotometer, Instrumentation Laboratory (UK) Ltd., Birchwood Science Park, Warrington) following the manufacture`s instructions. Blank determinations were subtracted from sample readings and multiplied by 5 to give mg/l of the element EDTA extractable, in the sample. To express the result as mg/kg, the readings were then divided by the density of 2mm soil [i.e. 0.05 x weight of 20g soil (air-dried, less than 2mm diam.)].

To measure *metals in plant material*, cadmium, copper, lead and zinc were determined by mixed acid digestion (Ministry of Agriculture, Fisheries and Food,

1986). Concentrated nitric acid (48ml) and perchloric acid (60%w/v) (12ml) were added to 5g of an oven-dried ground sample of plant material in a 250ml conical beaker, covered and left overnight. The contents were digested slowly over moderate heat (100°C, for 2h). The temperature was increased to 180-200°C and the excess nitric acid allowed to evaporate, reducing the contents to approx. 5ml and until the appearance of white fumes. The temperature was increased further until all the perchloric acid was volatilised and the beaker contained a dry residue. After cooling, 5ml of HCL (6M) was added to the beaker, and the contents simmered for 5min., After cooling 20ml of water was added and the digest was filtered (Whatman No. 44). Blank digests were carried out in the same way. The samples were stored at 4°C before being read by atomic absorption, as above. Standard solutions of each metal 0-20ng/ml cadmium 0-200ng/ml copper; 0-200ng/ml lead; 0-150ng/ml zinc were prepared in 0.3M HCl (Spectrosol standard solutions, [1000ppm], BDH).

Titanium, zinc, cadmium, copper and lead analyses of plant material were carried out independently by A.D.A.S., Wolverhampton, England, using the same extraction methods, to check for possible soil contamination of plant material (Ti) and verify results (Mitchell, 1960; Bowen, 1974).

For *coal tar* analysis, oven-dried soil samples (10g) were extracted in a Soxhlet apparatus with 85ml toluene for 4h. The filtered extract (17ml) was evaporated on a boiling water bath and the residue weighed. If the weight was greater than the equivalent of 5mg/g of sample, then gc-ms was used to identify the major polyaromatic hydrocarbons present in the coal tar.

Polyaromatic hydrocarbon (PAH) analysis of Derwenthaugh soil was determined using gas chromatography-mass spectrometry (gc-ms). A sample of oven-dried soil (50g) was placed in a pre-weighed cellulose thimble (Whatman, double thickness) and extracted in a Soxhlet apparatus with dichloromethane:methanol 97:3 for 72h. Any elemental sulphur present was removed by the addition of 5g copper turnings

(previously washed in HCl (5M) until a salmon pink colour, washed in distilled water 6 times, methanol twice and solvent twice). If the copper turned black, more was added. The filtered extract (Whatman No. 40) was concentrated by rotary evaporation at 30°C until approximately 25ml were left. Glass plates (20 x 20cm) were thoroughly washed before use with hot detergent solution (Decon 90), rinsed in distilled water and dried. The silica gel layer (0.5mm thick) was prepared from an aqueous slurry of Merck Kieselgel type 60G and 70ml distilled water. The wet coatings were allowed to dry 2h before the plates were activated overnight and stored in an oven at 120°C. Prior to use, the plates were cleaned by development with ethyl acetate and then reactivated (120°C for at least 1h).

The sample, containing 25μg solids dissolved in 100μl dichloromethane), was applied as a band about 2cm from the bottom of the plate. To increase separation of the aliphatics and light aromatics from other compounds present, the plate was run in dichloromethane for the first 2cm above the origin then, when dry, fully developed in light petroleum. The different bands were visualised by spraying the plate with a methanolic solution of Rhodamine G and then viewing under ultraviolet light. On each plate a reference standard, a mixture of n-hexadecane [n-alkane],1-phenyldecane [mono-aromatic] and phenanthrene [tri-aromatic] was used. Four bands corresponding to the R_f values of the standards were removed and called aliphatics (ALI); light aromatics (LARO); aromatics and some polar compounds (HARO); nitrogen, sulphur, oxygen, polar compounds and spot line (NSO).

The bands were scraped off the plate and then the hydrocarbons were removed from the silica gel by desorption with 50ml of light petroleum/dichloromethane in the following ratios; aliphatics (40:10); light aromatics (25:25); aromatics and some polar compounds (0:50); nitrogen, sulphur, oxygen, polar compounds and spot line, dichloromethane/methanol (45:5); using a short column containing a bed (1cm depth) of alumina. After removal of most of the solvent by rotary evaporation, the elutates were transferred to pre-weighed vials, evaporated to dryness and weighed. 100μl solvent/mg

dry wt. extract was added to each vial. No more than 1μl was injected into the gc for gc-ms analysis.

The Perkin-Elmer 8420 gas chromatogram (Perkin Elmer, Beaconsfield, Bucks, UK) was fitted with a non-polar OV-1 fused capillary column (10m length) and with on column injection and a flame ionization detector. The temperature was programmed to run from 100 to 240°C at 5° per minute. Samples were injected at ambient temperature and the injector immediately ballistically heated to 300°C. Helium was used as the carrier gas at a flow rate of 5.0 ml/min..

The gc-ms system consisted of a Hewlett Packard 5890 linked to a 5970 mass selective detector controlled by a series 9000 216 chem station. Samples were acquired using split-splitless injection on an HP5 column (5% phenylmethyl silicone, 25mm x 0.2mm I.D., 0.11μm film thickness), in full scan mode.

While analysis by gc had much to offer in terms of resolution of mixtures and sensitivity of detection of individual components, compound identification was severely hampered by masking, co-elution and clatheration. However some identifications were possible. The functionality of the unknown compound or compound series was indicated by thin layer chromatography (i.e. the saturated, non-saturated, mono aromatic or aromatic fraction). Most compounds were identified using computerised gc-ms. The recognition of compounds from their individual mass spectra was made by comparison with standard/published spectra or by spectral interpretation. Mass fragmentography was also used to characterise homologous series and to aid compound identification.

2.5 Plant growth experiments

2.5.1 Treatment of pots

Plastic pots (15cm diam) used in greenhouse experiments had a disc of filter paper placed at the bottom of each pot. They were filled with a proprietary soil compost or river Tyne dredgings and placed in plastic saucers to enable watering to be carried out by sub irrigation. This avoided the loss of elements by leaching and obviated the need for overhead watering which would have contaminated the barley leaves by soil splashing. Three seeds were sown per pot and the pots were arranged in a randomised block.

2.5.2 Construction of outdoor plots

10 trial plots, (0.5m x 1m) were constructed at Moorbank Experimental Garden, Claremont Road, Newcastle-upon-Tyne. Railway sleepers (12cm depth by 25cm wide) were securely wired and nailed together to confine the wet dredgings. As the plots were sited on a heavy clay soil, drainage channels (15cm deep by 7.5cm wide) were cut in each plot and pipes laid to assist drainage (Bramley, 1985) (Photograph 3). The plots and sleepers were covered in heavy duty black polythene to avoid contamination from the sleepers and seepage between plots. The base of the polythene was perforated, as moisture content dictated that the dredgings would probably be anaerobic and consequently microbial degradation limited. Coarse gravel (4cm depth) was laid on the polythene base to aid drainage, increase aeration and hence promote (phenolic) degradation.

To improve soil texture, straw or spent mushroom compost were added to some of the plots. Additional nitrogen was also added to these plots to compensate for the removal of nitrogen in the decomposition process (Wild, 1988). The organic material was spread over the dredgings (20:80 by volume) and dug in, giving a final depth of dredgings in all plots of 23cm. Nitrogen was added at two levels, 15g N m^{-2} for all

Photograph 3. **Pictorial view of construction of outdoor plots at Moorbank Experimental garden, Claremont Road, Newcastle.**

plots and a higher one (two-fold) to the plots with the organic material. For simplicity, two levels of a 20:10:10 fertiliser was used rather than a mixture of fertilisers. To summarise, the plots, arranged randomly, were as follows:

1) Dredgings x4
2) Dredgings + Straw (clean, pesticide and weed-free) x2
3) Dredgings + Spent Mushroom Compost x2
4) Arable, pesticide-free (control) Soil x2

Nitrogen was applied in March and April, 1988 and again in March and April, 1990. After each application the plots were cultivated by spade and fork thoroughly to aerate the soil and facilitate the even distribution of nitrogen. The pH of the plots was tested prior to sowing.

Lettuce, *Lactuca sativa* L. var. Butterhead was grown, during 1988, on outdoor plots of Tyne dredgings (0.5m x 1m) in drills 30cm apart, at a depth of 1cm. Seedlings were thinned out at 14 days to 30cm apart. Growth rate (plant diameter (cm) at 14, 21 and 28 days) and yield were determined.

The plots were staked out and marked with string and the barley was sown in drills 15cm apart. The seeds were 2.5cm deep. The two outside rows were used as `guard rows` leaving five test rows in each plot. The plots were sown with barley in May, 1988 and May, 1990. The first crop was harvested in August, 1988; the second in August, 1990. In September 1989, lime (calcium hydroxide) was added to four plots of the river dredgings (2 dredgings, 1 straw, 1 spent mushroom compost) to raise the pH from 6.7 (average) to between 7.1 and 7.3.

Weed invasion during the experimental first year was minimal on the dredged material but considerable on the arable soil - such weeds were removed by hand. By the third year the number and species of weeds colonising the dredged material had increased, but chickweed, (*Stellaria media*) the predominant weed in the arable plots, was not one of them.

2.5.3 Seeds

Spring barley (*Hordeum vulgare*, variety Kym) was chosen as the main experimental crop. A named cultivar was considered to be the most appropriate plant because of its consistent genotype. Barley was chosen because its long roots would make it more susceptible to pollutants leached from the top few centimetres of the soil and accumulated below and because of its sensitivity as a pollution indicator.

2.5.4 Measurement of emergence, growth rate and biomass yield

The percentage emergence of the seeds was calculated by determining the number of plants successfully emerged and raised in each plot. Growth rate was determined by measuring the extension of the first leaf of each plant 10 days after seed germination and again at 17 and 24 days. The growth rate (mm/day) of each plant was calculated and then the mean rate for the plants in each pot or row per plot determined. Approximately three months after sowing (when the plants on the arable control soil were beginning the reproductive stage of growth), the whole plant including the root was harvested carefully to minimize damage to the root. Excess soil was shaken off and the plant roots were washed under running tap water and rinsed in double distilled water before recording the fresh weight per row. Plants in pots were cut at soil level and the aerial portions of the plants were harvested, and transported in paper bags (Mitchell, 1960). All handling of plants was made using plastic disposable gloves to avoid contamination of the leaves. The plants were dried to a constant weight (100°C for 18h) and the mean dry weight per pot or per row was recorded. Mature leaves from below the growing tip on main stems were collected and cut up with a pair of stainless steel scissors, ground in a mill and dried again at 60°C. Subsamples were then taken for metal analysis (Jones, 1971).

One-way analysis of variance (ANOVA) (Sprent, 1977), with significance selected at a 5% level of probability, was performed on the data from plant growth

experiments.

2.5.5 Soil extraction

A proprietary soil compost was spiked with zinc ($ZnNO_3$), cadmium ($CdNO_3$), copper ($CuSO_4$) or lead ($PbNO_3$) to verify percentage recovery when extracted with EDTA (ammonium ethylenediaminetetra-acetate). Zn, Cd, Cu or Pb solutions (0.5-300mg/kg) were added to 30g compost (air-dried, sieved to less than 2mm) in Pyrex dishes and mixed thoroughly in a blender (for 1min, slow speed). Each treatment was replicated three times. The dishes were stored in glass jars, to maintain moisture content, for 24h. Samples were extracted with EDTA as indicated in Section 2.4. Three blanks were run simultaneously. Recoveries were consistent with the amount of metal added to the compost. There was a 97-100% recovery at the three concentrations tested for all metals and variation between replicates was less than 0.7% (Table 7).

2.5.6 Growth of rye-grass on River Tyne dredgings

Pots (7.5cm diam) containing dredgings or soil compost were sown with rye-grass, *Lolium perenne* L. var Melle at 50 seeds per pot. The pots, in triplicate, were watered every other day and left in a growth room (day length 16h, achieved with fluorescent lighting), temperature 19°C, for 3 weeks. Whole plants were harvested and dry weights recorded.

2.5.7 Growth of barley on River Tyne dredgings and soil compost with different levels of fertiliser

Hydrofertiliser 52 regular (20:10:10 nitrogen: phosphorus: potassium), a multi-functional fertiliser, was applied to soils in triplicate, at the following levels of nitrogen; none; 3.5g; 7.5g; 15g and 22g of N (all per m^2).

Table 7. Recovery of metals (EDTA available) from soil compost spiked with zinc, cadmium, copper and lead nitrate.

	metal conc. (ppm)	metal extracted from spiked compost (ppm)*	Recovery (%)
Zn	100	101	101
	150	148	99
	200	200	100
Cd	0.5	0.51	101
	1.0	1.05	105
	2.0	1.99	99
Cu	10	10.3	103
	20	19.5	97
	40	40.6	101
Pb	50	50.0	100
	100	99.8	100
	200	204	102

*value (mean of 3 replicates) with background concentration of 1.5ppm ± S.E. 0.01 subtracted. Standard deviation less than 7%

2.5.8 Growth of barley on River Tyne dredgings and soil compost mixes

Dredgings (air-dried, sieved 2mm) were mixed with soil compost in the following ratios (by vol.): 100:0 75:25 66:33 50:50 33:66 25:75 0:100.

2.5.9 Replanting experiment

A replanting experiment was introduced to determine the effect on root length and yield of barley grown, firstly, on soil compost for 4 weeks and replanted in Tyne dredgings for a further 8 weeks and secondly, on Tyne dredgings for 4 weeks followed by soil compost for 8 weeks. The trial was carried out in triplicate. Undisturbed controls were also included.

Spring barley (*Hordeum vulgare* L. cv Kym) (3 seeds/pot) was planted in soil compost or River Tyne dredgings. Four weeks after planting (a quarter way through the life cycle) plants growing in soil compost were lifted and replanted in River Tyne dredgings, while those grown on the River Tyne dredgings were uplifted and replanted in soil compost. Control plants growing in soil compost were uplifted and replanted in soil compost again. Root length at 4 weeks and yield at harvesting (12 weeks) were recorded.

2.6 Microbiological analysis

2.6.1 Viable counts

Viable counts of bacteria in Derwenthaugh (DWH) soil were measured by the improved recovery methods introduced by Hopkins *et al.*, (1991). Soil (5g fresh weight) was mechanically blended (Kenwood Chefette) for 1min (medium speed) with 10ml of 0.1% (w/v) sodium cholate solution (Sigma). The liquid was transferred to a 50ml centrifuge tube with the addition of a further 10ml sodium cholate solution, 10ml of iminodiacetic acid chelating resin in the Na^+ form (Sigma) and approximately 30 glass beads (diam 1.5mm) and shaken end-over-end (Stuart Scientific Rotator Drive STR4) at speed 10 for 2h at 5°C.

Samples were centrifuged (Beckman HS8) at 500g (2033 rev/min) for 30sec., the supernatants pooled and the pellet resuspended in various solutions as detailed in Table 8. The soil residue was discarded and the combined pool of supernatants from each sample were centrifuged for 10min at 5000 rev/min and the supernatants discarded. The residue was made up to 5ml with 0.8% (w/v) saline. Serial dilutions of the final residue were made with saline and 0.2ml of the dilutions were plated onto nutrient agar, nutrient:water (bacteriological No. 1) agar 1:5 and water agar containing 2mM *p*-cresol and 1mM potassium thiocyanate, and left to incubate at 23°C.

2.6.2 Zurich Test

The Co-ordinating European Council (CEC) developed the Zurich test (CEC L-33-T-82) for evaluating the biodegradability of test lubricants against that of standard calibration materials, for example, natural vegetable oils and (mineral) white oil. It was adapted for use with either two-stroke cycle outboard engine oils, or exhaust condensate materials in water but it is applicable with any water-immiscible organic compound. As the Derwenthaugh soil was known to contain much organic material, derived from coal carbonisation, a sewage inoculum, an inoculum from Derwenthaugh

Table 8. Experimental procedure for viable counts

Mechanically blend 5g fresh wt. soil with 10ml 0.1% (w/v) sodium chlolate solution. Transfer to centrifuge tube with the addition of a further 10ml sodium cholate solution, 10ml Na^+ form chelating resin and approx. 30 glass beads. Shake for 2h at 5°C

↓

Centrifuge at 500g for 2min. Keep supernatant

↓

Resuspend pellet in 10ml Tris buffer pH 7.4 and shake for 1h at 5°C

↓

Centrifuge at 500g for 1min. Keep supernatant

↓

Resuspend pellet in 20ml sodium cholate solution and mildly ultrasonicate for 1min. Add a further 10ml sodium cholate solution and shake for 1h at 5°C.

↓

Centrifuge at 500g for 1min. Keep supernatant

↓

Resuspend pellet in 10ml Tris buffer pH 7.4 and shake for 1h at 5°C

↓

Centrifuge at 500g for 1min. Keep supernatant

↓

Resuspend pellet in 30ml distilled water and shake for 1h at 5°C

↓

Centrifuge at 500g for 2min. Keep supernatant

↓

Resuspend pellet in 30ml distilled water and shake for 1h at 5°C

↓

Centrifuge at 500g for 2min. Keep supernatant

↓

Centrifuge combined supernatants at 5000 rev/min for 10min, make up to 5ml in 0.8% saline. Discard residue.

↓

Prepare serial solutions of the combined supernatants and plate 0.2ml samples.

enrichment and CCl_4 extracts of the Derwenthaugh soil were used to examine the biodegradability of its lipophilic material.

The mineral media for the Zurich test was prepared by taking 1ml of each of the solutions A,B,C and D And diluting to 1 litre with double distilled water.

Mineral media

Solution A: (g/l)	
KH_2PO_4	8.50
K_2HPO_4	21.75
$Na_2HPO_4.2H_2O$	33.40
NH_4Cl	15.00
Solution B: (g/l)	
$MgSO_4.7H_2O$	22.50
Solution C: (g/l)	
$CaCl_2.2H_2O$	36.40
Solution D: (g/l)	
$FeSO_4.7H_2O$	3.00
$ZnSO_4.4H_2O$	0.10
$MnSO_4.4H_2O$	0.50
$CuSO_4.5H_2O$	0.05
$CoSO_4$	0.01
H_3BO_3	0.75
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.05

Flasks containing mineral media (75ml) were set up as follows:

Incubation time time days	test flasks per test oil	poisoned test flasks per test oil	calibration flasks	poisoned calibration flasks	neutral flasks
0	3	-	3	-	2
7	3	-	2	-	-
14	3	-	2	-	-
21	3	2	3	2	-

Neutral flasks were made up with 75ml of mineral media and 1ml of the inoculum but with no C-source. Test flasks were prepared as for the neutral flasks with the addition of 25μl of an extract of the DWH soil in carbon tetrachloride. Calibration flasks were prepared as for neutral flasks with the addition of 25μl of a solution of rape seed oil in carbon tetrachloride (15g/100ml). Poisoned flasks (containing test or calibration material) were prepared as for either test or calibration flasks but without the inoculum. Mercuric chloride solution (1ml of a 1g/100ml solution) was added to poison each flask. This determined abiotic loss.

The sewage inoculum was prepared from approximately 1 litre of raw sewage obtained no more than 2h previously from the Sewage Works of Northumbria Water at Riding Mill, nr. Hexham, Northumberland. The Derwenthaugh inoculum was grown in batch cultures on cresol (3mM, mixed isomers). Sewage and batch cultures of Derwenthaugh inoculum were centrifuged for 1h at 1200 revs/min, the supernatant discarded, and the remainder centrifuged for 30min at 1200 revs/min. Again the supernatant was removed and the remainder centrifuged for 10min at 1200 rev/min. The bacterial cells in the final sewage suspension were counted using a haemocytometer and if numbers exceeded 10^6, used as an inoculum (0.5ml) for the test and neutral flasks. The flasks were incubated on an orbital shaker (180 rev/min) at 25°C for 7, 14 and 21 days.

After incubation, the contents of the flasks were poured into a 500ml separating funnel, with ungreased joints. The flask was rinsed with 10ml CCl_4 which was then poured into the separating funnel. The funnel was shaken for 2min by hand and left to stand, to allow the CCl_4 phase to separate out. The oil content was determined by i.r. spectroscopy of the CCl_4 fraction.

The mean value of the i.r. absorption of the neutral flasks was obtained and subtracted from i.r. absorption of zero day flasks. The mean value (X_E), standard deviation and co-efficient of variation of zero day flasks were determined. The later

being less than 5%. The residual oil content of calibration, test and poisoned flasks (E_{flask}) was then calculated using the following equation:

$$\text{Residual oil content (\%)} = \frac{E_{flask} - E_{neutral\ flask}}{X_E} \times 100$$

$$\text{Biodegradability (\%)} = \frac{P - T}{P} \times 100$$

where P = Residual oil content of poisoned flask in % (mean values)

and T = Residual oil content of test flasks in % (mean values)

2.6.3 Bacterial enrichment

The growth medium used for liquid culture was a modification of the mineral salts medium used by Witton (1988).

Composition of mineral salts media

Stock A : (g/l)

Na_2HPO_4	60.0
KH_2PO_4	30.0
NaCl	5.0
NH_4Cl	10.0

Stock B: (g/l)

$MgSO_4.7H_2O$	24.6
$CaCl_2$	1.1

Trace elements:(g/l)

$FeSO_4.7H_2O$	0.45
$MgSO_4.7H_2O$	24.6
$CaCl_2.2H_2O$	1.5
$ZnSO_4.7H_2O$	0.144
$MnSO_4.4H_2O$	0.112
$CuSO_4.5H_2O$	0.025
$CoSO_4.7H_2O$	0.028
H_3BO_3	0.006
HCl (conc)	5.13

For normal concentration - 100ml A, 1ml B and 1ml trace elements was made up to 1

litre and adjusted to pH 7.0. When potassium thiocyanate was used as the nitrogen source, NH_4Cl was omitted from the mineral salts medium. The liquid minimal media were sterilised by autoclaving for 20min at 101.3 KPa. Both carbon and nitrogen sources were filtered sterilised using a membrane holder and receiver (Nalgene) with Whatman cellulose nitrate filters (47mm diam, 0.45μm pore size), before being added aseptically to the previously sterilised growth media. Solid media was prepared by the addition of 1.2% (w/v) Bacteriological agar No.1 (Oxoid) to the mineral salts medium with cresol as the carbon source and potassium thiocyanate as the nitrogen source. The agar plates were allowed to cool to approximately 45°C after autoclaving before the addition of the carbon/nitrogen sources prior to pouring (20ml agar per plate). Nutrient agar (Oxoid 2.8%w/v) and mixtures of nutrient agar and bacteriological agar No.1 (1% w/v) in a ratio of 1:5 were also used as solid growth media to characterize organisms in viable counts and during identification.

Soil extract was prepared using the method by James (1958). A high fertility soil (500g) in 1500ml tap water was autoclaved for 30min at 121°C, filtered through sterile muslin, sealed in a sterile container and stored at 5°C for later use.

2.6.4 Enrichment of methylated phenol-degrading organisms

Enrichment cultures were set up using 2,4,6-trimethylphenol, 3,4,5-trimethylphenol and commercial cresol (*ortho*, *meta* and *para* isomers, mixed) as the sole carbon source. Tyne river dredgings, coke and gas work waste soil (1g) or Tyne water (10ml) was added to 50ml of mineral salts medium in 250ml Erlenmeyer flasks supplemented with trimethylphenol (0.5; 1.0; and 2mM) and cresol (2mM). These enrichment cultures were incubated for 72h at 25°C in Gallenkamp Orbital Incubator Shakers. Three subcultures steps were performed to isolate and adapt a mixed bacterial culture to cresol before inoculation into a chemostat for further enrichment (Harder, 1981).

A chemostat, L.H. 500 series modular fermenter with an operating volume of 900ml (LH Fermentation Ltd. Slough, UK) of the type described by Baker (1968) was used for continuous enrichment. The mineral salts medium, with cresol (2mM) as the sole carbon source, was pumped into the vessel by a peristaltic pump (Watson Marlow HR flow inducer UKC B1310) via a break tube. A constant culture volume was provided by means of a weir overflow tube which was also the gas outlet. The culture was agitated, at a speed of 800 rev/min by a Rushton type impeller which, together with an air intake (1.0ml/min) provided vortex aeration. This gave sufficiently good aeration to allow satisfactory culture of aerobes without oxygen becoming limiting. A 50ml batch culture inoculum was added aseptically to the fermenter to initiate growth. The continuous culture was run at a dilution rate of $0.06h^{-1}$. Steady state was achieved after three volumes of medium had been pumped through the chemostat. The temperature was maintained at 20°C and the medium was buffered at pH 7. Different dilution rates, D, were obtained by altering the flow rate, F, of the medium. The concentration of cresol was increased in 1mM steps every 10 days up to 6mM.

Growth of the bacteria (in liquid culture) was monitored by measuring the absorbance of the growth media at 540nm in a Beckman DU-7 spectrophotometer. The dry weight of a cell suspension was determined by filtering 1ml of the culture through a pre-weighed Whatman cellulose nitrate membrane filter (20mm diam., 0.4μm pore size). Residual salts were removed by washing the filter with 2ml distilled water. Following this, the filters were dried overnight (38°C) and re-weighed.

The chemostat, fed with 6mM cresols was sampled daily over a period of weeks and when HPLC results indicated the continued absence of cresol in the culture, low levels of potassium thiocyanate (0.5mM) were added to the feed growth medium. The flow rate was kept constant. KSCN concentrations were analysed as follows: Aliquots (0.5ml) of the centrifuged supernatant were diluted with 9.5ml double-distilled water, 1ml of a solution of ferric nitrate solution [50g $Fe(NO_3)_3$ in 500ml distilled

water, with 25ml of conc. HNO_3, diluted to a litre] was added and the solution read at 480nm, using water as a blank. The results were read against a standard calibration curve of 0-500μg/ml KCNS (Stafford & Calley, 1969; A.P.H.A., 1983).

2.6.5 Cresol extraction and analysis

The amount of cresol in the culture fluid was determined on samples, from which bacteria had been removed by centrifugation in a Microcentaur centrifuge, (1200 rev/min for 10min) and suitably diluted, by direct spectrophotometric analysis at 207nm. Uv wavelength scans of cresol standards had indicated maximal absorbance at this wavelength. Analysis and quantification were achieved using an LDC/Milton Roy HPLC system (LDC/Milton Roy, Stone, Staffs., UK) equipped with a Merck-Hibar Lichrosorb RP-18 column (250 x 4.0mm int. diam.). The system encompassed a Constametric 3000 solvent delivery system, a Spectromonitor 3000 variable u.v. wavelength detector and a C1-10 B computing integrator. A Gilson model 231 Autosampler was also fitted. The mobile phase employed was acetonitrile:water 6:4 (by vol.) and the flow rate was 1.0ml/min. Methyl phenols in the column effluent were monitored with a variable wavelength UV detector set at 207nm.

The retention times for the cresols were as follows: *m*-cresol, 3.27min; *p*-cresol, 3.72min; *o*-cresol, 4.41min (Fig. 12). The extraction method described consistently gave recoveries greater than 99% (104±4.5) over a range of cresol concentrations (1-10mg/litre). Cresol concentration was quantified using standard solutions of cresol in acetonitrile:water (5:5 by vol.) mixtures. All compounds investigated gave a linear calibration over the range tested (0-2.7mg/l).

2.6.6 Biochemical assays

Amino-nitrogen (Moore & Stein, 1954)

The ninhydrin reagent was made up as follows: anhydrous sodium acetate,

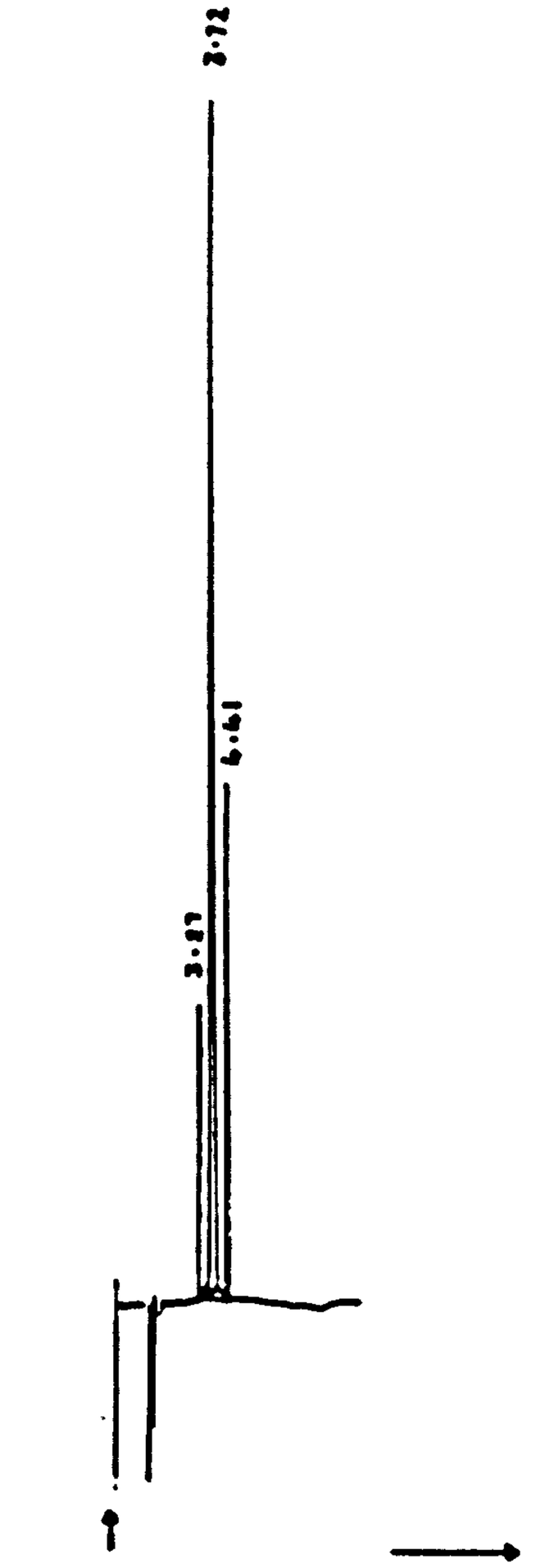


Figure 12. HPLC Trace of Cresol

After centrifugation of culture supernatant (200 rev/min for 10min), cresol was analysed using an LDC/Milton Roy HPLC system equipped with a Merck-Hibar Lichrosorb RP-18 column (250 x 4.0mm int. diam.) and a u.v. detector set at 207nm. The mobile phase employed was acetonitrile:water 6:4 (by vol.) and the flow rate was 1.0ml/min. The retention times for the cresols were as follows: *m*-cresol, 3.27min; *p*-cresol, 3.72min; *o*-cresol, 4.41min.

45.6g was dissolved in 100ml water; glacial acetic acid, 12.5ml, was added, followed by 100ml methoxyethanol. Ninhydrin (indonetrionehydrate) 6g, was added to the solution and when dissolved, 100ml of 2-methyloxymethanol was added, followed by stannous chloride, $SnCl_2$ (0.25g) and the solution made up to 500ml with water. The liquid was transferred to a dark bottle and nitrogen gas was bubbled through for several hours until the liquid was a pale straw colour. The quantitative ninhydrin reagent (1ml), ascorbic acid (0.07% w/v), (1ml) and the sample of culture supernatant, (1ml) were mixed and heated in a boiling water bath for 10min. The absorbance was then read at 570nm. The concentration of nitrogen in the supernatant was measured by reference to a calibration curve prepared with ammonium chloride (0μg/ml-100μg/ml).

Sulphate was determined using Bertolacini & Barney`s chloranilate method as modified by Johnston *et al.*, (1975). Culture supernatant (1ml) was added to potassium hydrogen phthalate (0.12M in 50% aqueous ethanol) (1ml). Solid barium chloranilate (3-5mg) was added and the suspension was vigorously agitated on a vortex mixer. The suspension was centrifuged to remove unreacted barium chloranilate, and the chloranilic acid in the supernatant was measured by its absorbance at 332nm. The concentration of sulphate was calculated by reference to a calibration curve prepared with $Na_2SO_4.12H_2O$ in the same manner.

Sulphite in the growth media was measured using the Ellman reagent method (Johnston *et al.*, 1975). Culture supernatant was allowed to stand in air for 20min to remove interference from thiols. A solution of 5,5`-dithiobis(2-nitrobenzoic acid) (1mg/ml in 0.1M potassium phosphate buffer, pH 7.0) (1ml) was added to 1ml of culture supernatant from which the bacteria had been removed by centrifugation and absorbance was read at 415nm. Sulphite concentration was obtained by reference to a standard curve prepared from freshly made solutions of $Na_2SO_3.7H_2O$.

2.6.7 Identification of chemostat organisms

Serial dilutions to 10^{-3} were made from the chemostat consortium using 1/4-strength Ringer`s solution as the diluent and 0.5ml of these dilutions were plated onto nutrient agar. The plates were incubated at 25°C for 48h, the appearance and growth of the colonies studied and samples Gram-stained.

The API 20 NE rapid identification kit for non-enteric Gram-negative rods was used as an initial aid to identification.

Further identification was done by the extraction and analysis of fatty acid methyl esters (Jantzen & Bryn, 1985). Biomass from an agar plate, was grown up in 2 x 500ml nutrient broth cultures, harvested, washed and freeze dried. Three replicates of biomass (50mg) were placed in 8.5ml glass tubes with 3ml of methanol:toluene:sulphuric acid (6:3:0.2), air was expelled by nitrogen, the PTFE caps sealed and the samples were left overnight in a heating block at 75°C. After cooling, the methanolysate was concentrated by nitrogen, a NaCl solution added (50% saturated, 2ml) and fatty acid methyl esters were extracted with petroleum ether. Light petroleum ether (2ml) was added to each tube and mixed for 15min on an end-over-end shaker. The contents were centrifuged for 5min at 1000rev/min and the top layer (petroleum ether) removed to a glass vial. This step was repeated and the petroleum ether extracts pooled. The petroleum ether extract was passed through a 2cm column of ammonium hydrogen carbonate followed by 2ml of diethyl ether and a further 1ml of petroleum ether and the eluent collected, pooled and evaporated under nitrogen (below 40°C). The samples and appropriate standards were spotted onto an analytical t.l.c. plate (alumina-backed silica gel) and onto a preparative t.l.c. plate (plastic-backed silica gel) and run in light petroleum ether:acetone (95:5). The analytical plate was sprayed with molybdo-phosphoric acid (5% w/v in ethanol) and left in the oven (70°C) for 15min. A charred spot near the top of the plate indicated the presence of the fatty acid methyl esters

(FAMES). The preparative plate was sprayed with Rhodamine 6G (0.1%w/v in ethanol), dried and viewed under u.v. light. The FAMES were cut out and placed in 8.5ml glass tubes containing diethyl ether (5ml) and extracted by rotating for 30min. The ether solution passed through a non-activated aluminium oxide column (2cm) followed by 1ml of diethyl ether. The solvent was evaporated under nitrogen below 40°C and analysed by a Perkin-Elmer 8420 gas chromatogram (Perkin Elmer, Beaconsfield, Bucks, UK) fitted with a non-polar OV-1 fused capillary column (10m length) with on-column injection and a flame ionization detector. The temperature was programmed to run from 100 to 210°C at 4°/min. After 15min at 210°C the temperature was increased to 240°C at 20°C/min where it remained for 5min. Samples were injected at ambient temperature and the injector immediately ballistically heated to 300°C. Helium was used as the carrier gas at a flow rate of 5.0ml per min. Eluted peaks were quantified using a Trio computing integrator (Trivector Systems International Ltd., Sandy, Beds., UK) and identified by equivalent chain length calculations (Gillan, 1983) by comparison with standard saturated even-numbered straight chain fatty acids (C_{10}-C_{20}, Sigma Chemical Co. Ltd., Poole, Dorset, UK). Standards and unknowns were run in triplicate.

2.6.8 Growth of cresol and thiocyanate cultures on catechols

Inocula from chemostat cultures were transferred to freshly-prepared culture flasks containing filtered-sterilised catechol, 3-methylcatechol, and 4-methylcatechol (2mM) with 1mM KSCN and mineral salts solution. Both dead cell controls and uninoculated controls were included. Flasks were incubated in the dark on an orbital shaker at 25°C and sampled over 85h. Catechol disappearance was measured by HPLC at 207nm. Retention times for catechol was 2.58min; 3-methylcatechol, 2.90min; and 4-methylcatechol, 2.78min.

2.6.9 Degradation of cresol and related methylphenols by cell suspensions of the chemostat mixed culture

To determine the metabolic versatility of the chemostat culture the oxidation (Section 2.6.10) and the degradation of a number of compounds was investigated by washed suspensions in turnover experiments. Exponential phase cells, grown on *p*-cresol (3mM) plus thiocyanate (1mM) were harvested by centrifugation (10,000 rev/min for 20min., Beckman J2-21 centrifuge). The cells were washed twice in phosphate buffer (25mM, pH 7.0) and resuspended in fresh phosphate buffer to give an OD_{540} of 1.0, from an aliquot of which the dry weight was determined. Aliquots of cell suspension (1ml) were dispensed into triplicate Erlenmeyer flasks (250ml) containing 95ml of minimal medium. Thiocyanate (1mM) from a stock solution, (filter-sterilised) was added to each flask. Cresol, dimethyl- or trimethylphenols were added from freshly prepared, filter-sterilised stock solutions to give a final concentration of 3mM. Because of the poor solubility of di- and trimethylphenols, test chemicals were first dissolved in 0.1M NaOH, followed by neutralisation to pH 7.0 with HCl and then made up to volume in a 10ml volumetric flask. Stock solutions were normally prepared at concentrations of 10mg/ml.

For zero-time samples the flasks were sampled (1ml) immediately on addition of the substrate and the cells removed by centrifugation (10,000 rev/min. for 5min., MSE Microcentaur). An aliquot of the supernatant (0.3ml) was removed, diluted with acetonitrile:double distilled water (50:50) and analysed by HPLC as described previously (Section 2.6.5). The HPLC system resolved all of the compounds tested. The experimental flasks were incubated at 25°C on an orbital incubator (200 rev/min) for the time course of the experiment. Control flasks containing media without inoculum were also included.

2.6.10 Measurement of oxidation rates of cresol and related methylphenols by whole cells.

Measurements of oxygen consumption by cell suspensions of the mixed chemostat and organisms `C` and `D` isolated from it (see section 2.6.7) in the presence of a range of methylphenols, were made using a Clarke oxygen electrode system (Rank Brothers, Bottisham, Cambridge, UK) linked to a chart recorder (Kipp & Zonen BD 40).

The electrode reaction chamber was maintained at 25°C by circulating water from a constant temperature water bath. The instrument was calibrated on air-saturated buffer in the reaction chamber, giving an effective 100% oxygen saturation, and zeroed by adding sodium dithionite crystals which react rapidly with the dissolved oxygen to provide an anaerobic situation and full scale deflection of the recorder. The cell suspension (0.5ml) prepared as in Section 2.6.9 was added to 2.5ml phosphate buffer in the reaction vessel and the background oxidation rate measured for 2min. If the substrate was added in dimethyl formamide (DMF) the endogenous oxygen uptake rate on addition of the appropriate amount of DMF was also determined. Substrate (50mM, 30μl equivalent to 1.5μmol) was added to the reaction vessel and the new rate of oxidation measured over 5min. The endogenous oxidation rate was subtracted from this to give the oxidation rate of substrate by whole cells. At 25°C the oxygen concentration of the air-saturated buffer was assumed to be 0.237 μmol/ml (Chappell, 1964) which was used in calculations of oxygen consumption rate.

2.6.11 Calculation of rate constants

In biodegradation experiments the rate constant (k) for each set of data was used to determine rates of substrate loss rather than expressing such loss as a percentage of that of the original amount after a given time. Plots of $\log_{10}$ (% substrate remaining) against incubation time were drawn up for each set of data and regression

analysis carried out to determine the slope k, (where the slope is -k/2.303 for $\log_{10}$ [substrate] v time) and the correlation co-efficient between the points (r) (Price & Dwek, 1979). Rate constants are expressed in $days^{-1}$ (Appendix 1).

2.6.12 Biodegradation of phenolics in Derwenthaugh soil

The incubation apparatus (Haigh, 1989) consisted of clean acid-washed, round Pyrex dishes (80mm diam. x 40mm depth) holding the soil samples (30g); the dishes, 5 in all, were stacked on stainless steel supports inside glass jars (30.5cm depth x 13.5cm breadth) with screw-top lids (Fig.13). Haigh (1989) found that the addition of an absorbent paper wick standing in water still did not prevent weight loss due to water evaporation from a variety of soils compared to experiments without the wick. Therefore, the maintenance of constant moisture levels in soil during incubation was carried out by adding water every 2-3 days to experimental dishes to return samples to their original fresh weight. Bottles of distilled water (pH 7.0) for this purpose were kept at incubation temperature. To mitigate any changes in soil moisture content of individual dishes, caused by moisture gradients formed within the jar, the position of the dishes was rotated every time water was added.

Nitrogen, phosphorus and potassium were added as mineral supplements to the soils in the form of Hydrofertiliser 52 regular - a commercially-available 20N;10P;10K fertiliser. The pellets were ground to a fine powder, weighed and mixed into each dish of soil, at a rate sufficient to supply additional nitrogen equivalent to 15g N m^{-2}.

Cells were grown in chemostat and in batch cultures on cresol (3mM, mixed isomers) and thiocyanate (1.5mM), harvested by centrifugation (Beckman J2-21, 1000 rev/min for 20min), washed twice and resuspended in tap water at 4°C. This suspension was diluted to a cell density sufficient to give an application rate of 10^6 bacterial cells/cm^2. Biomass was added at 10^6 organisms/g soil.

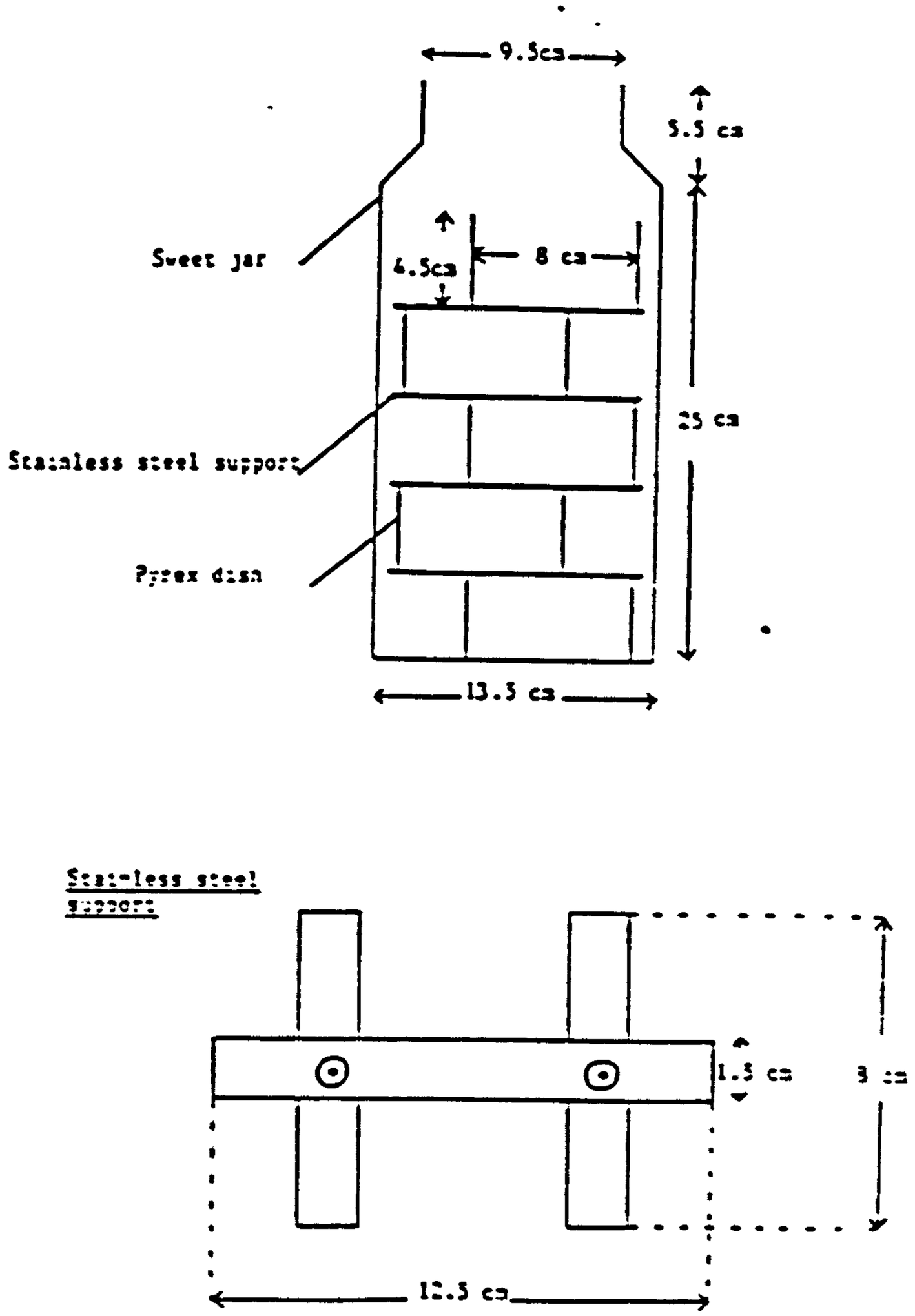


Figure 13. Incubation apparatus used for monitoring the loss of phenols and total hydrocarbons from soil taken from a former coke work site at Derwenthaugh, Blaydon, nr. Newcastle-upon-Tyne.

2.6.13 Development of an extraction protocol for phenols in contaminated soil

Phenol analysis of gas/coke work wastes generally involves an alkaline extraction of the soil to release phenols bound to the soil particles (Wilson & Stevens, 1981), distillation of the re-acidified extract to remove non-volatile impurities followed by colorimetric analysis using 4-aminoantipyrene (A.P.H.A., 1983). However as it was considered possible that the more volatile phenols in the contaminated Derwenthaugh soil could be lost during the distillation process, after alkaline extraction, the acidified aqueous phenolic extract was extracted with an organic solvent before analysis by HPLC. Any non-phenolic organic compounds that had the same retention time as phenolic compounds and would interfere with the results would be removed by the alkaline extraction. The possibility of interference by esters was considered but thought negligible due the history of the site. Secondly, the 4-aminoantipyrene colorimetric test was not considered suitable for analytical use because it failed to determine those *para*-substituted phenols where the substitution is an alkyl, aryl, nitro, benzoyl, nitroso or aldehyde group, for example *para*-cresol (Emerson, 1943).

The method eventually was as follows; Soil (30g) was shaken with 50ml sodium hydroxide (1M) for 24h, the mixture filtered and the residue washed with a further 20ml sodium hydroxide and the pooled alkaline extracts acidified to pH 7.0 with HCl (1M). The aqueous solution was extracted three times with a total of 100ml diethyl ether (GPR) using a 250ml separating funnel. To determine the cost effective number of extractions needed, the aqueous solution was extracted into ether up to four times with a backwash before the extractant was analysed. 3-Fold extractions with backwash were carried out as concentrations in the fourth extraction were insignificant. The mixture was swirled for 2min by hand and the phases allowed to settle. Because an emulsion usually formed at the interface, 5g (approx) NaCl was added to aid separation of the ether layer, the solution swirled gently and the solution left to settle for at least 15min. The ether layers from successive extracts were pooled into a volumetric flask,

to which 0.5g (approx.) anhydrous Na_2SO_4 was added and left for 2h to dry. The ether extract was concentrated using an unheated rotary evaporator and the residue was redissolved in 50:50 acetonitrile:water prior to centrifugation at 1200 rev/min for 10min and HPLC analysis at 270nm. The sum of the five phenolic compounds was used in biodegradation experiments (Fig.14). Identification was based on retention times of two columns with different liquid phases using authentic reference standards of a range of methylated phenols. *ortho*-Cresol, 3,4-dimethylphenol, resorcinol and 2,4,6-trimethylphenol were identified as four of the five peaks, the other one remained unidentified.

The efficiency of the method of phenol extraction described above was tested by spiking the DWH soil with additional phenol at three different concentrations in triplicate and determining phenol recovery. Phenol alone (no soil) was used at the same three concentrations as a control. Air-dry soil (30g) was weighed into a Pyrex dish. Phenol (0.25; 0.5; 1.0mM) in water (0.5ml final volume) was added to the dishes in triplicate and mixed thoroughly. Soil with water added, acted as one control and water with phenol as the other. Dishes were placed in the incubation apparatus and left for 10h at 25°C. The extraction process as detailed above was performed. The mean amount of phenol recovered after extraction was plotted against the amount of phenol added to soil before extraction for both control and sample dishes (Fig 15). The graph follows the equation;

$$y = mx + c$$

where, y = absorbance and, x = the phenol concentration

$$\% \text{ recovery} = \frac{\text{slope of [phenol + soil]}}{\text{slope of [phenol alone]}} \times 100$$

A recovery of 87% was obtained (Fig. 15).

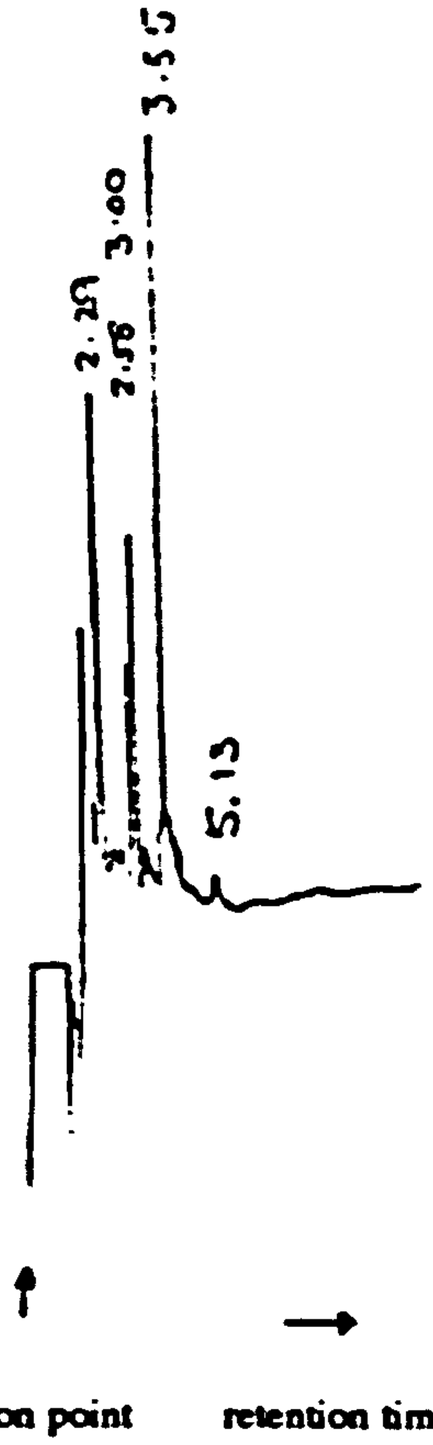


Figure 14. HPLC trace of phenolic compounds from phenolic extraction of Derwenthaugh soil.

Soil (30g) was shaken with 50ml sodium hydroxide (1M) for 24h, filtered and acidified to pH 7.0 with HCl (1M). The aqueous solution was extracted three times with 100ml diethyl ether (GPR). The ether extract was concentrated and analysed by HPLC. An LDC/Milton Roy HPLC system was used, equipped with a Merck-Hibar Lichrosorb RP-18 (250 x 4.0mm int.diam.) column. The mobile phase employed was acetonitrile:water 6:4 (by vol.) with a flow rate of 1.0ml/min. Phenolic compounds in the column effluent were monitored with a variable wavelength u.v. detector set at 270nm. The peak at retention time 3.0.min was identified as *ortho*cresol; 2.29min as resorcinol; 3.55min as 3,4-dimethylphenol and 5.13 as 2,4,6-trimethylphenol. The other peak remained unidentified.

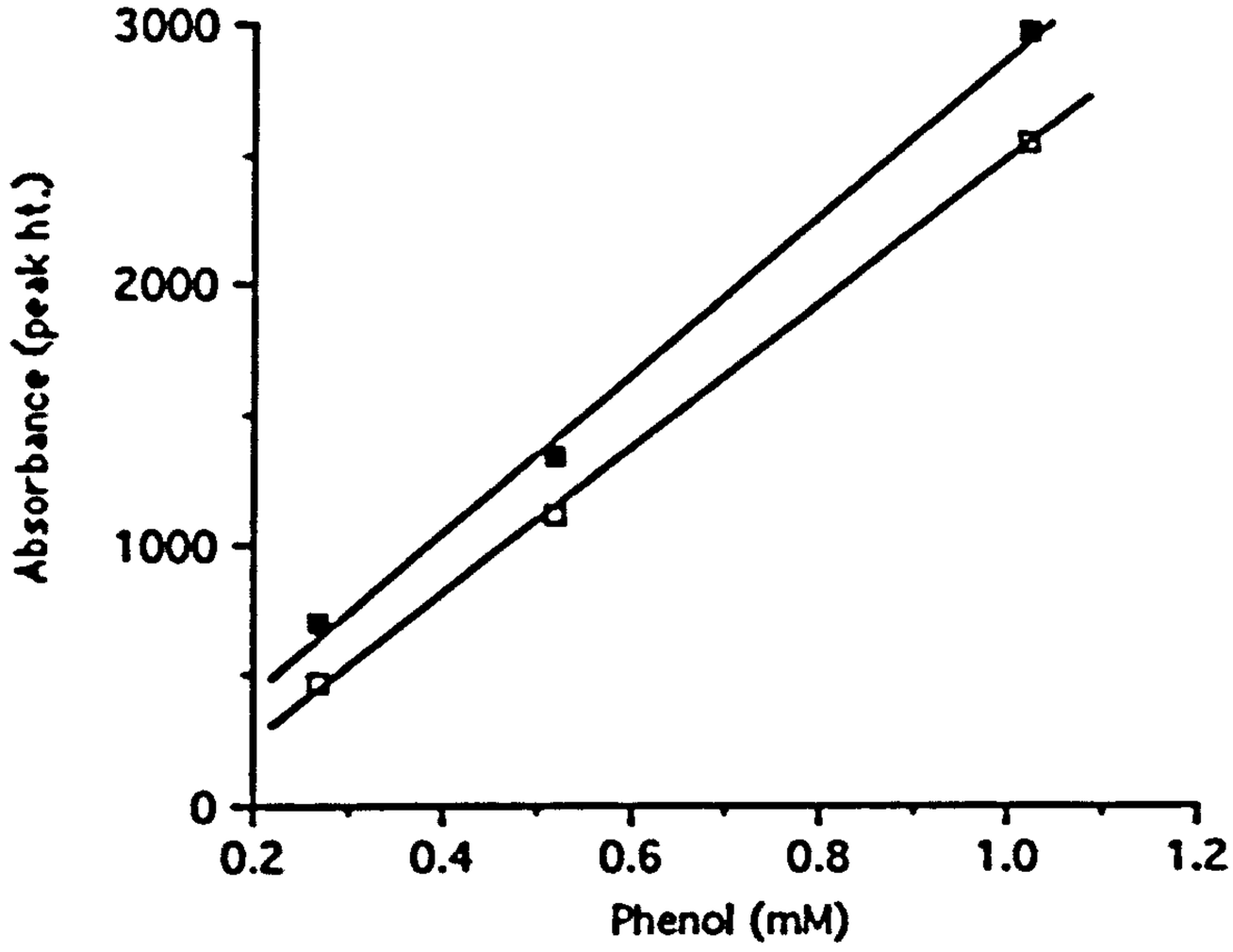


Figure 15. Percentage recovery of phenol from Derwenthaugh soil.

Derwenthaugh soil (30g) was spiked with phenol (0.25;0.5; 1.0mM), shaken with 50ml sodium hydroxide (1M) for 24h, filtered and acidified to pH 7.0 with HCl.The aqueous solution was extracted with diethyl ether, concentrated and analysed by HPLC. Phenol was extracted by the same process.

The lines were derived by linear regression.

Derwenthaugh soil □ (r) correlation = 0.994.

Phenol ■ (r) correlation = 0.997

2.6.14 Addition of laboratory grown biomass or arable soil to DWH soil as an inoculum

Preparation of soil for this experiment was carried out by placing 500g DWH soil (prepared as above) in a large plastic basin with 500g compost. The two soils were mixed thoroughly by hand and quartered before weighing out 30g of the mixed soils into each of five dishes for each experimental condition, as follows:

DWH soil pH 5.5

DWH soil pH 7.0

DWH soil pH 7.0 + biomass (1 x 10^6g/dry wt.)

Arable soil pH 6.5

DWH soil + arable soil (50:50w/w) pH 7.0

All dishes, except DWH soil pH 5.5, were immediately supplemented with Nitrogen:Phosphate:Potassium (20:10:10) mix. One set of dishes were further supplemented with biomass addition (10^6 organisms/g soil) and yeast extract (Oxoid, 0.1% w/v) as Ellis *et al.*, (1990) had shown that nutrients added as N, P and organic supplements optimized degradation of organically-contaminated soil. Zero time dishes were then analysed. The remaining dishes were incubated, moisture levels kept constant and the soil mixed by stirring by hand every 2-3 days and the position of the individual dishes in the incubation jars rotated to eliminate the possible effect of a moisture gradient occurring within the jars. The jars were incubated for 2 months during which time soil was extracted at 2, 4, 6 and 8 weeks for phenolic analysis. When substantial degradation of the contaminating phenols in these soils had taken place in the dishes with biomass additions, a *p*-cresol (1mM) supplement was added to this set of dishes and its biodegradation further monitored.

RESULTS

3.0 Experiments with Tyne dredgings

3.1 Introduction

180 acres of industrially contaminated waste land (including a former gas works site) along the River Team from Redheugh to the Team Valley Trading Estate was remediated and transformed into gardens for the Gateshead Garden Festival 1990 and subsequently for residential use.

One of the attractions was the marina at Dunston Staiths. In order to construct the marina, several hundred tons of silt were dredged to deepen the river basin. Rather than disposing of the silt at sea, at an estimated cost of £800,000 the dredgings were used as a soil substitute for much of the site. A horizontal barrier (1.5m thick) of coarse aggregate and sand was laid over the contaminated land. The dredgings were used to cover the barrier and as a base for the mounding for the extensive flower displays the festival required.

Dredged material from rivers such as the Tyne, which run through industrial areas, are generally polluted. A study of metal levels in Tyneside soils by Aspinall *et al.* (1988) found that the distribution of Pb, Cd and Zn contaminated soils was closely associated with shipbuilding, heavy engineering and chemical industrial works. These activities were concentrated along parts of the River Tyne waterfront from the beginning of the eighteenth century until the late 1960`s. Therefore, it seemed probable that material dredged from Dunston Coal Staiths would be polluted with i) Pb, Cd and Zn ii) run-off from the adjacent Redheugh gas works site and iii) coal residues from coal loading over a 90 year period.

Apart from problems of organic and heavy metal contamination, dredged material is usually characterised by the predominance of silt sized particles (2-63μm) which would increase the water-holding and working properties of the river dredgings. As the dredgings were also from the tidal reach of the river, they would be expected to show high salinity .

To remedy the extremely high moisture and salt content together with the total lack

of structure, a period of "ripening" has been generally recommended. During this process the water content decreases through evaporation, and cracks appear, allowing aeration of the sediment (De Nekker & In`t Veld, 1975). One year has been advocated (Brown *et. al.*,1980), though Rimmer (1985) showed that the problems of excess water and high salinity in landed dredgings were relatively short term (10-20 weeks) as long as the depth of dredging material did not exceed 1m.

Prior to the dredging operation, Gateshead Metropolitan Borough Council commissioned the analysis of 18 samples from three boreholes that were sunk into the sediment in the Dunston basin. The contractors who collected the borehole samples described the material as a `very soft black organic silty clay`. Subsequent particle analysis by Rimmer (1985) showed the material was of a silty clay loam texture comprising 70% silt with 23% clay and 7% sand. The report by the Public Analyst (Pattinson, 1984) revealed the dredgings were contaminated with metals, in particular As, Pb and Zn and with organic materials associated with the former gasworks including coal tar derivatives and phenols. High concentrations of phenolic compounds (5-120mg/kg) were found in all 18 sites sampled. The "threshold trigger values" (see Introduction p12) set out in the ICRCL Guidance on the Assessment and Redevelopment of Contaminated Land (DOE, 1987) for phenol is 5mg/kg and land with a value above this is deemed unsuitable for either landscaped or domestic garden use.

Rimmer`s report (1985) on the landed dredgings also showed the cation exchange capacity of the dredgings was high, reflecting the large amount of clay and organic matter. This indicated the potential for the dredgings to hold phytotoxic metals in readily available forms, such as exchangeable ions. Thus the River Tyne dredgings were thought to be toxic due to the presence of a combination of both organic compounds and heavy metals.

3.2 Analysis of dredged material

The Tyne river dredgings had been landed and allowed to weather for approximately one year before the present investigations began. The dredgings were of a very fine material (silty clay loam) with high water retention capacity. The pH of the dredgings was 6.7 compared with 6.5 of an arable soil measured at the same time and the percentage loss on ignition of the dredged material was 7.8% compared with 8.6% for the arable soil. Results of around 9-10% loss on ignition are common for arable soils.

Analysis of the dredgings indicated that contaminants associated with former coal carbonisation sites, including phenolic compounds, were all below the `threshold trigger values` (Table 9). Mean concentrations of available Zn (490mg/kg), total Cd (7.5mg/kg) and total Pb (510mg/kg) were above `threshold trigger values` (Table 10). Other metals were all below `threshold trigger values`. Available Cu (58mg/kg), although below `threshold trigger values`, was monitored in plant growth experiments as a previous ICRCL paper indicated levels above 50mg/kg may be phytotoxic (Smith, 1981). Independent verification of available metals in dredged soil was obtained from the M.A.F.F. Analytical Laboratory, Leeds. Their single sample analyses fell within 10% of my determinations.

3.2.1 Infra-red analysis of dredged material

Infra-red spectra of the dredgings, examined as a solid dispersion in KBr discs or as a thin film in CH_2Cl_2 (both transparent in infrared), are shown in Figures 16 & 17. At the high frequency end of the spectrum, the peaks occurring between 3696 and 3620cm^{-1} indicate the presence of -O-H or N-H vibrations (Fig. 16). The cluster of bands around the 3000cm^{-1} indicate the presence of hydrocarbons, as expected from coal residues. The peaks at just over 3000cm^{-1} are due to unsaturated hydrocarbons while those just under at 2922 and 2852cm^{-1} (Fig. 17) occur in the region of saturated stretching. The exact positions indicate the asymmetrical and symmetrical stretches respectively for

Table 9. Analyses of River Tyne dredgings.

Physical properties	Dredgings	Arable
Water content (%w/w)*	19	9.0
Loss on ignition (%w/w)*	7.8	8.6

Contaminant	Conc. of contaminant (mg/kg soil dry wt.)*	Threshold trigger values**
pH	6.7	5
Phenol	4.5	5
Polyaromatic hydrocarbons+	42	50
Cyanide (free)	<1.0	25
Thiocyanate	9.0	50
Sulphate	1900.0	2000
Sulphide	6.0	250

* Values were the mean of at least three determinations

** Threshold trigger concentrations (mg/kg air-dried soil) for contaminants associated with former coal carbonisation sites for use in domestic gardens (DOE, 1987)

+ This figure represents the extractable material by hot toluene extraction. Since this concentration was below the threshold value of polyaromatic hydrocarbons (PAHs), no further analysis was undertaken.

Table 10. Metal analyses of River Tyne dredgings

Element*	concentration of metal ion in soil (mg/kg)+	
	Arable soil	Tyne dredgings
Zinc (available)	16.5	490.0
Zinc (total)	69.0	1200.0
Cadmium (available)	0.33	2.6
Cadmium (total)	0.5	7.5
Copper (available)	6.6	58.2
Copper (total)	18.0	140.0
Lead (available)	14.0	210.0
Lead (total)	63.0	510.0
Arsenic (available)	0.1	11.6
Arsenic (total)	1.0	5.0
Nickel (available)	2.3	3.8
Chromium (available)	0.2	1.2

* Total metal analyses by W. Stelling, Geography Dept., University of Newcastle-upon-Tyne.

+ Standard deviations of the three values <5% of mean.

Threshold trigger concentrations (mg/kg air-dried soil) for domestic gardens (DOE, 1987)

Cadmium (total)	3
Lead (total)	500
Arsenic (total)	10
Zinc (available)	300
Copper (available)	130
Nickel (available)	70

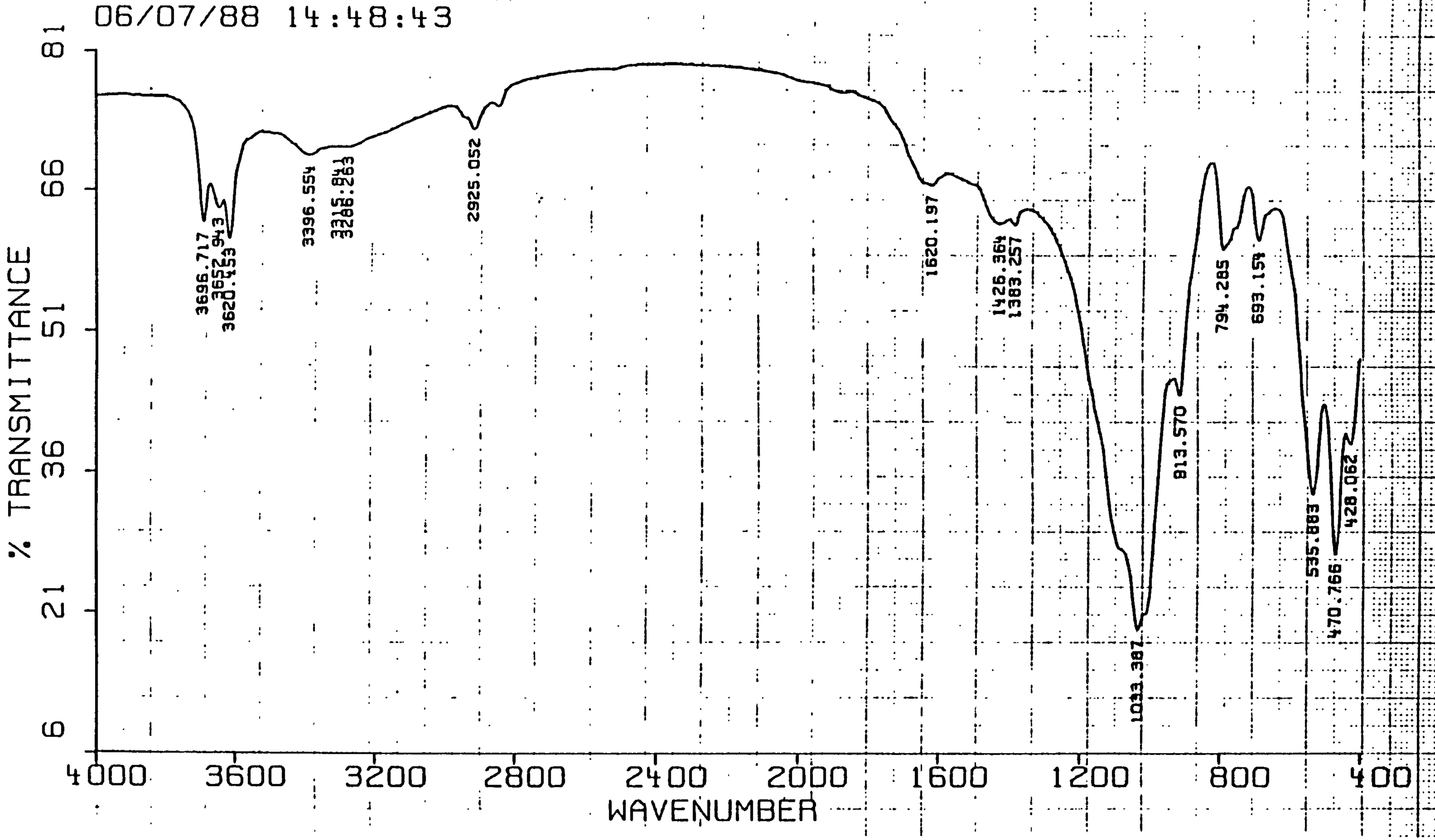


Figure 16. Infra red scan of River Tyne dredgings in KBr disc.
A sample of sieved (>2mm) oven-dried river Tyne dredgings was run as potassium bromide admixed disc (ratio of sample to KBr = 1:300), on a Nicolet 20 PCIR.

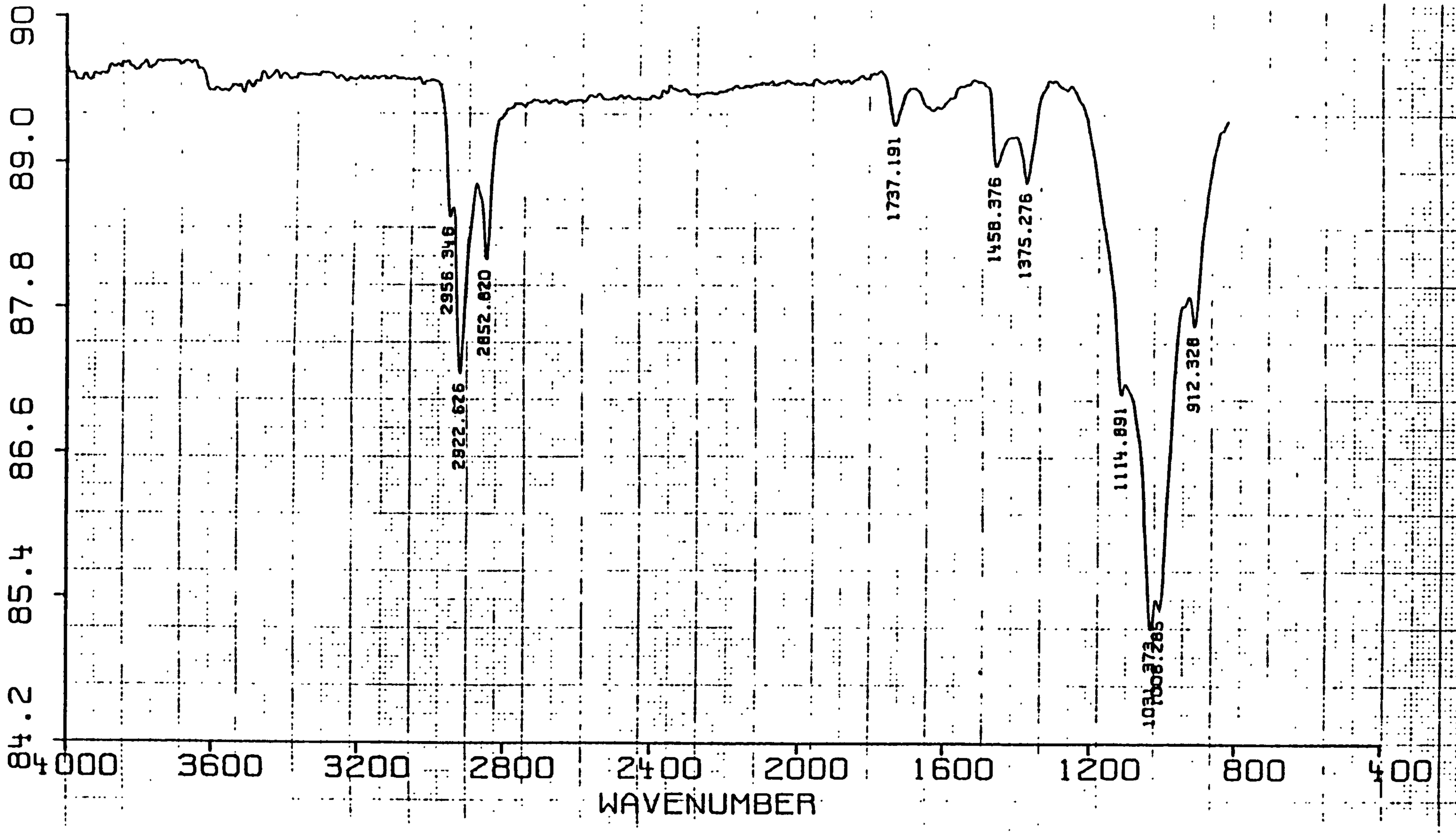


Figure 17. Infra red scan of River Tyne dredgings in CH_2Cl_2 extract.

A sample of sieved (>2mm) oven-dried river Tyne dredgings was run with a dichloromethane extract on a Nicolet 20 PCIR.

methylene groups. Absorption due to phenols appear between 1400 and 1170cm^{-1}, but the bands at 1383 and 1426cm^{-1} (Fig. 16) (or 1458 and 1375cm^{-1}, Fig. 17) could also be attributed to methyl groups in aliphatic hydrocarbons, as absorption at these frequencies are associated with the symmetrical and asymmetrical deformation of methyl groups. As saturated C-H stretching between 3000 and 2800cm^{-1} are present the latter interpretation is probably more likely. When an i.r. spectrum of silica, SiO_2 (Fig. 18) was superimposed on that of the dredged material the peaks at 1033cm^{-1} and 428cm^{-1} matched well, highlighting the high content of clay present in the river dredgings. The SiO_2 spectrum had been run in Nujol (a mixture of alkanes) so certain peaks had to be substracted from the spectrum obtained. Three main spectral regions have been used in the analysis of aromatic structures. These are: near 3000cm^{-1} for the C-H stretching absorption, the 1600-1500cm^{-1} group, where aromatics give one peak near 1600cm^{-1} and another just below 1500cm^{-1}, and 700-900cm^{-1} range where patterns associated with ring substitution occur. If hydrogen is directly attached to the aromatic rings, absorption should occur in each of the above 3 regions whatever the type of aromatic compound. The spectra of the dredged material indicated absorption in these 3 regions although the 700-900cm^{-1} region overlapped with the SiO_2 spectrum (Jones & Sandorfy, 1956).

In conclusion, analyses of the dredged material from the River Tyne had shown it to be of a silty clay loam (Rimmer, 1985), with the expected predominance of silt sized particles resulting in a fairly heavy, poorly aerated soil. Heavy metals - in particular Zn and Cd (though also Cu and Pb to a lesser extent) - were present at levels great enough to be a cause for concern. Analysis of organic residues showed that potential pollution problems from the adjacent Redheugh gas works were unfounded and although hydrocarbon residues were detected by i.r., these were low enough to present no problem of organic contamination. Phenolic compounds present originally in borehole samples were no longer above threshold trigger levels in the dredged material. It was thought that phenols initially present on landing were volatilised on exposure to air (or leached or degraded). Although

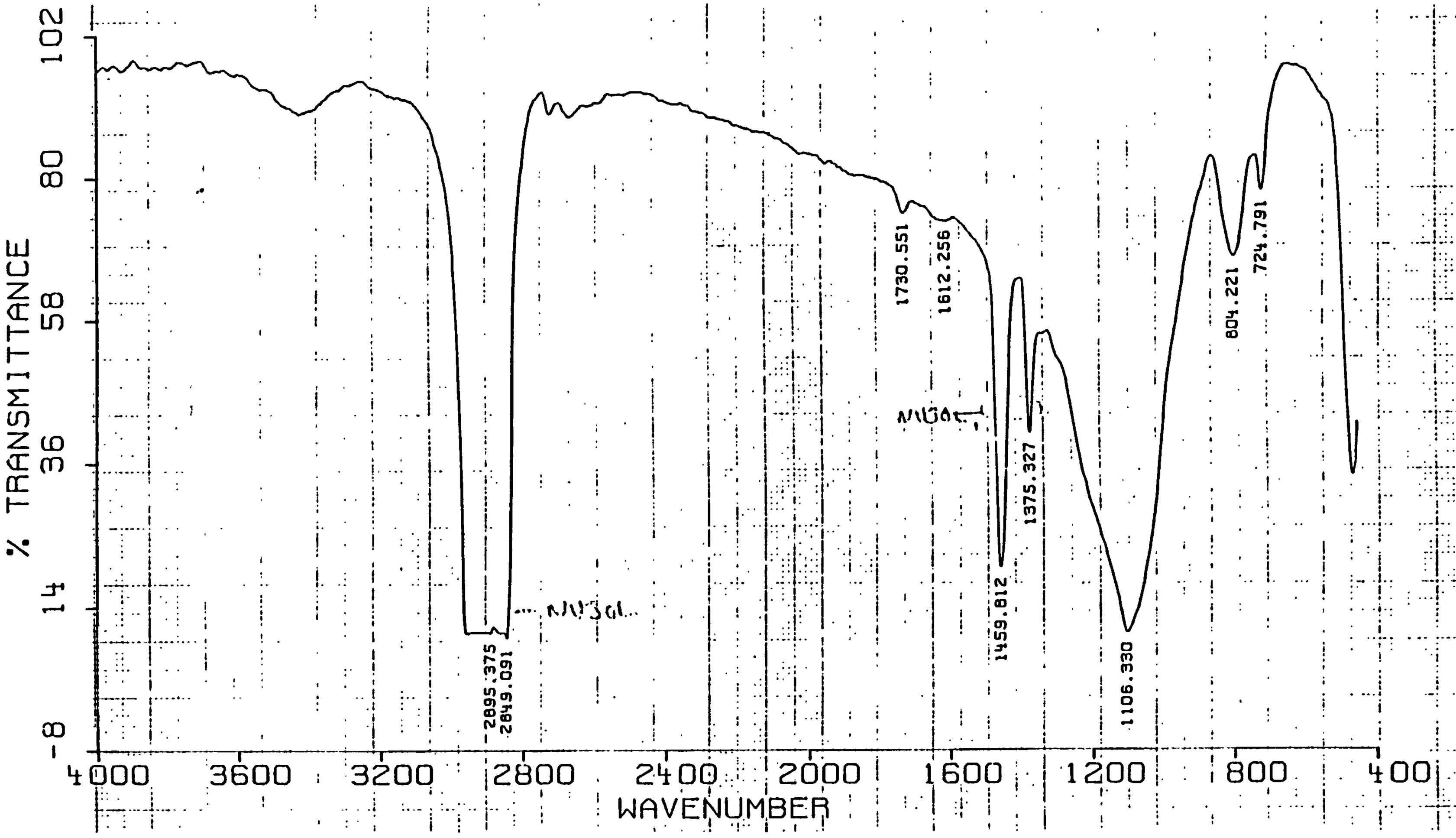


Figure 18. Infra red scan of silicon dioxide in Nujol.
A sample of silicon (IV) dioxide was run in Nujol on a Nicolet 20 PCIR.

work continued on the remediation of the River Tyne dredgings, a second site with phenolic contamination was also selected for remediation as the original aim of the project was the bioremediation of phenolic contamination by the introduction of bacteria previously selected from such a site.

3.3 Plant growth on the River Tyne Dredgings

The primary aim of this project was the remediation of the soil structure and heavy metal contamination so that the land spread river dredgings could be used for recreational amenities, gardens or possibly for agricultural productivity. The criteria on which the ameliorative treatments were judged were short and long term efficacy and cost. In the light of these criteria, general treatments possible were 1) addition of fertilisers, inert or organic material; 2) mixing with clean (uncontaminated) soil or 3) weathering.

It was thought that the weathered dredgings would be low in one or more nutrients therefore the dredgings were analysed for the major plant nutrients (i.e. nitrogen, phosphate and potassium). Soil analyses (ppm/g air-dry soil) indicated that (i) phosphate was already present in low amounts (20ppm in the dredgings compared with 62.5ppm in the arable soil); (ii) potassium was present in moderate amounts (220ppm in the dredgings compared with 437.5ppm in the arable soil); and (iii) ammonium-N was absent in the dredgings (0ppm compared with 35ppm in the arable soil). To a soil badly depleted in nitrogen with low to moderate levels of potassium and phosphate, MAFF (1988) recommended additions of NPK of 150:60:60 kg/ha for the growth of barley. To determine the most cost-efficient amount of fertiliser to apply to the river dredgings, a range of fertiliser levels from 0-220 kg/ha (0-22g $N\ m^{-2}$), in the form of Hydrofertiliser 52 regular, a 20:10:10 fertiliser mix, was applied to pots of barley sown in the river dredgings or in soil compost. In order to investigate the bioavailability of metals in the dredged material,

bioassays using barley (*Hordeum vulgare* L *cv* Kym), ryegrass (*Lolium perenne* L cv Melle), and lettuce (*Lactuca sativa* L. cv Tom Thumb) were carried out.

3.3.1 Growth of barley on the river dredgings with different levels of fertiliser

Barley growing on the river dredgings in pots (15cm diam.) with different levels of fertiliser all very quickly demonstrated typical heavy metal poisoning; plants had an unhealthy spindly appearance, leaf tips were brown and withered, and both growth rates and yields were poor (Fig. 19). When the results were analysed by analysis of variance (ANOVA), the level of fertiliser added to the pots of Tyne dredgings was found to have made no significant difference to the growth rate ($p > 0.05$). Mean yields of barley actually decreased arithmetically as fertiliser supplementation to the pots of Tyne dredgings increased over 15g N m^{-2}, although the difference was not statistically significant ($p > 0.05$) (Fig. 19).

In contrast, growth rates of barley grown in soil compost without fertiliser was significantly improved when 3.5-22g ammonium-N m^{-2} was added to the compost (Fig. 20). Mean yields increased in proportion to the amount of fertiliser added e.g. the mean yield of barley grown with 15g or 22g ammonium-N m^{-2} increased significantly ($p > 0.05$) compared to the mean yield of barley grown in an absence of fertiliser (Fig. 20).

In conclusion, MAFF-recommended level of 150kg/ha (equivalent to 15g m^{-2}) of ammonium-N was added to the River Tyne dredgings, arable control soil (hereafter known as control soil) and soil compost control in subsequent experiments. It was hoped that the previously mentioned methods of alleviating metal toxicity would allow the fertiliser to improve growth rates and yields of barley grown on the river dredgings.

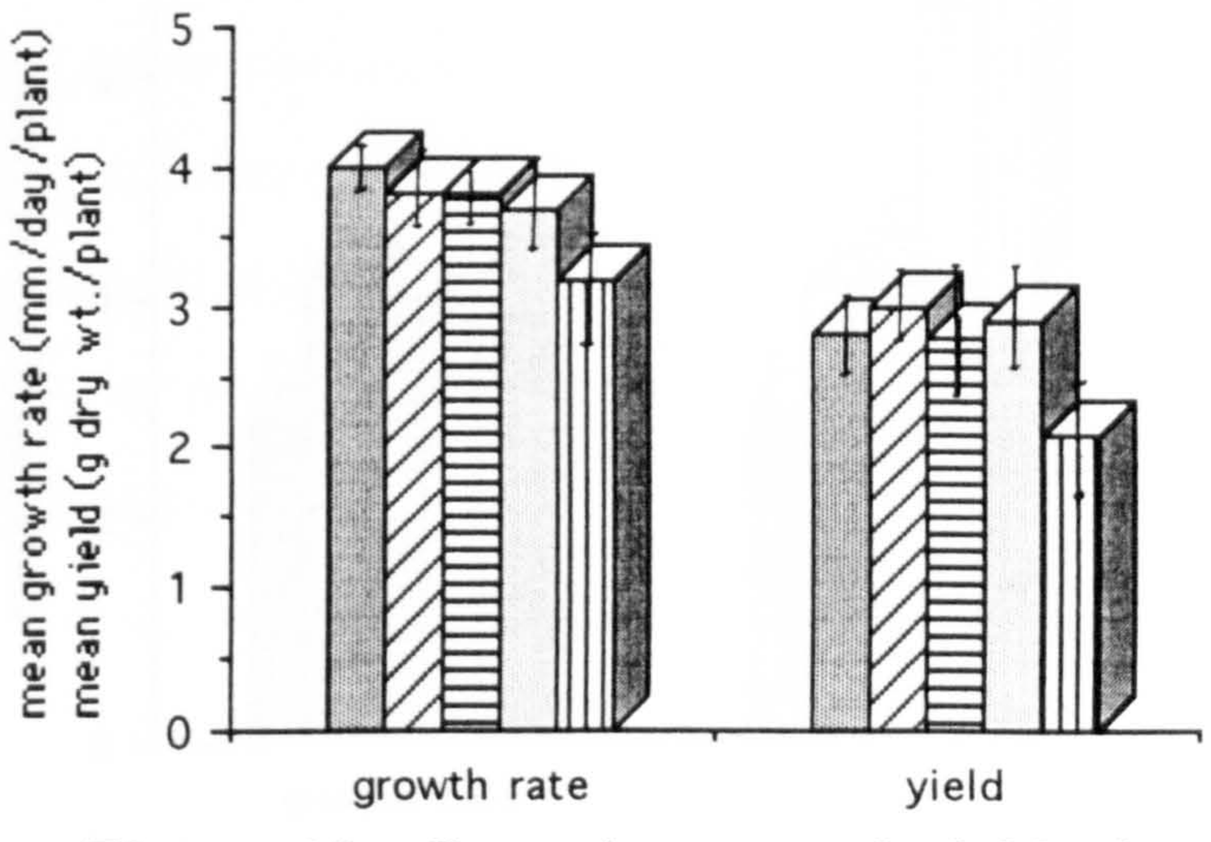


Figure 19. Growth rate and yield of spring barley grown in River Tyne dredgings with different application rates of nitrogen.

Spring barley (*Hordeum vulgare*) was grown under glass in 15cm diam. pots (3 plants/pot) of River Tyne dredgings with 0-22g N m^{-2} applied to pots, in the form of a 20:10:10 fertiliser mix; 0g ■; 3.5g ▨; 7g ▤; 15g ▩ and 22g ▥ N m^{-2}. The mean growth rate was determined by measuring the extension of the first leaf of each plant 10, 17 and 24 days after seed germination. Plants were harvested at maturity (approx. 3m after sowing) and mean dry weight exc. roots determined. Error bars indicate standard deviations.

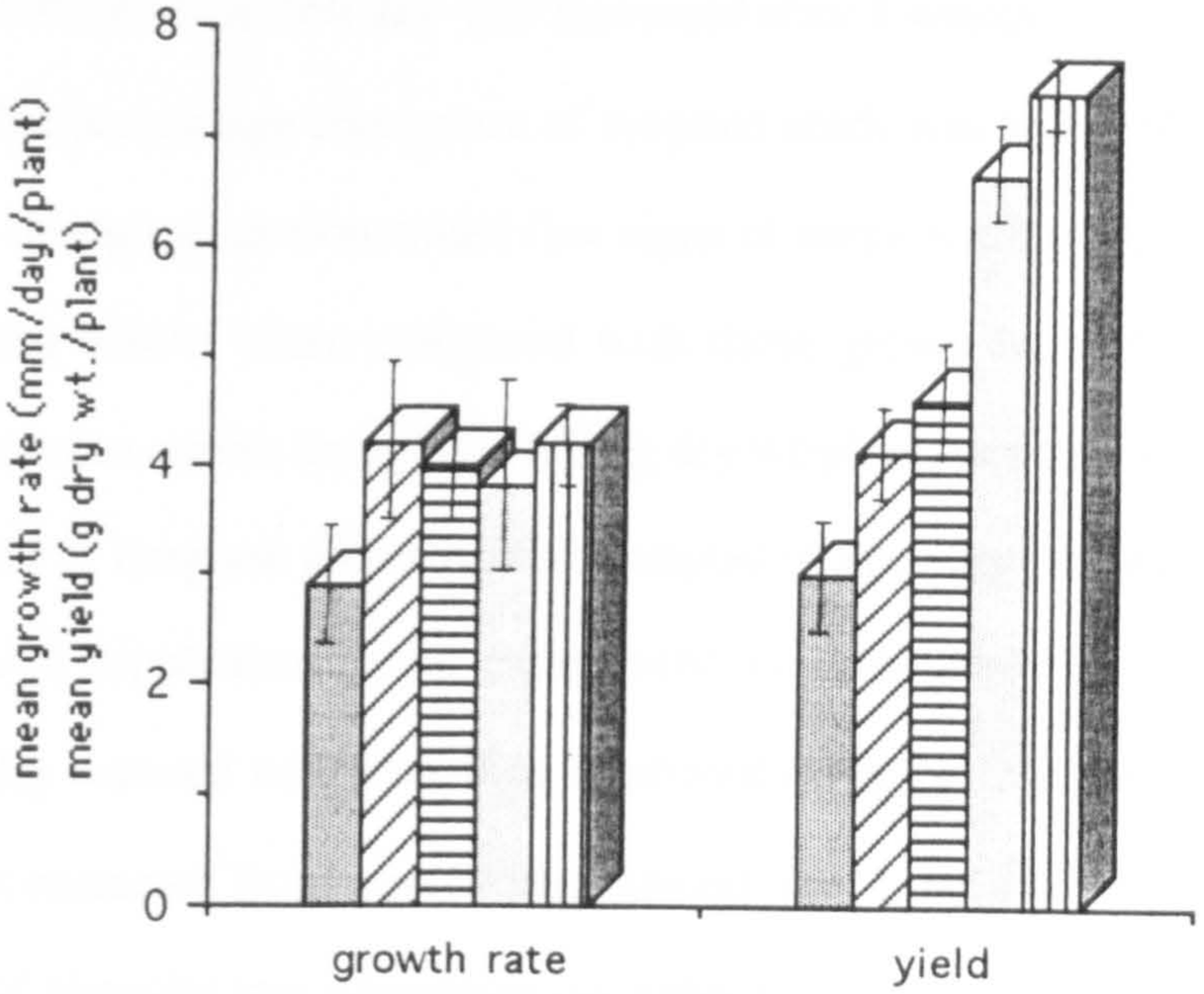


Figure 20. Growth rate and yield of spring barley grown in soil compost with different application rates of nitrogen

Spring barley (*Hordeum vulgare*) was grown under glass in 15cm diam. pots (3 plants/pot) in soil compost with 0-22g N m^{-2} applied to pots, in the form of a 20:10:10 fertiliser mix; 0g ■; 3.5g ▨; 7g ▤; 15g ⊡ and 22g ▥ N m^{-2}. The mean growth rate was determined by measuring the extension of the first leaf of each plant 10, 17 and 24 days after seed germination. Plants were harvested at maturity (approx. 3m after sowing) and mean dry weight exc. roots determined. Error bars indicate standard deviations.

3.3.2 Growth of rye-grass on river dredgings

Ryegrass was chosen initially for plant growth experiments because of speed of growth and ease of handling. Pots (9.5cm diam) of river dredgings (or soil compost as a control) were sown with *Lolium perenne* L cv Melle (50 seeds per pot), kept in a growth room at 19°C with an 16h day and harvested after 3 weeks.

The percentage emergence of ryegrass seeds was high (98-100%). Plants grown on the river dredgings demonstrated few signs of metal toxicity but leaves were of a slightly paler green colour when compared with those grown on soil compost. Mean yield of ryegrass grown on the dredgings (6.7mg dry wt/plant) was not significantly different from mean yield of ryegrass grown on soil compost (6.9mg dry wt./plant) (Table 11).

As a supplement to this experiment, ryegrass seeds were planted in pots as before but initially watered with a solution of phenol (5mg/kg; 10mg/kg) or a 5mg/kg solution of phenolics extracted from a soil contaminated with coke work waste (and representative of the type of phenolic mix contaminating industrial soils) to mimic the levels found originally in the dredgings. Emergence rates remained high (97-100%) and there were no visual symptoms of phenolic toxicity of rye-grass at these concentrations (Photograph 4). The plants grown in soil compost and watered initially with phenol demonstrated higher yields (mean 8.2±1.1mg dry wt./plant) than control plants (6.9±0.02) (Photograph 5). This was particularly noticeable with plants watered with 5mg/kg phenol (9.1±0.8) and less so with plants watered with phenolics (5mg/kg) extracted from Derwenthaugh soil or with 10mg/kg phenol (Table 11). Analysis of variance confirmed that the mean yield of rye-grass watered with phenolics was significantly higher than the mean yield of rye-grass watered without phenol, when grown on soil compost. Mean dry weights of ryegrass grown on dredgings and watered initially with phenols was not significantly different from the control, i.e. those watered without phenols.

Table 11. Effect of phenols on yield of Perennial Rye-grass.

Plastic pots (9.5cm diam.) containing River Tyne dredgings or soil compost were sown with perennial rye-grass (*Lolium perenne*) (50 seeds/pot). The pots were watered initially with phenol (BDH) at a concentration of 0.5mg/kg or 10mg/kg; or phenolics (0.5mg/kg) extracted from Derwenthaugh soil or distilled water. Plants were grown under fluorescent light (day length 16h) at 19°C and harvested at 3 weeks. Mean dry weights per plant were calculated.

Treatment	Mean dry wt./plant (mg)[=v] Compost	Dredgings
Control (water)	6.9	6.7
Phenol (10mg/kg)	8.6	6.1
Phenol (5mg/kg)	9.1 (±0.08)	6.8
DWH phenolics (5mg/kg)*	7.0	6.5

[=] Mean of three replicates

[v] Where the standard deviation of three values is <5% of mean, it is not included.

* Derwenthaugh (DWH) soil was extracted with sodium hydroxide followed by diethyl ether as in Section 2.6.13.

Photograph 4. (top) Perennial rye grass grown on River Tyne dredgings and watered with (L to R); water, 5mg/kg DWH phenolics, 5mg/kg phenol, 10mg/kg phenol.

Photograph 5. (above) Perennial rye grass grown on compost and watered with (L to R); water, 5mg/kg DWH phenolics, 5mg/kg phenol, 10mg/kg phenol.

3.3.3 Establishment of outdoor plots

Outdoor experimental plots were established to allow accelerated weathering of the dredgings. In this case dredging depth did not exceed 1m and dredgings were laid on a bed of gravel (4cm) on a base of perforated polythene over drainage channels in soil. The dredgings were dug over by hand at regular intervals to break up and further aerate the soil before organic material was added. Straw and spent mushroom compost were added to four of the ten plots, to encourage water movement through the dredgings, improve texture, increase microbial activity and reduce availability of toxic metals for plant uptake.

A basal supplement of 15g N m^{-2}, equivalent to the total yearly addition of nitrogen to a cereal crop such as barley of approximately 150kg per hectare, was added to all the plots (MAFF, 1988). Additional ammonium-N (15g m^{-2}, giving a total of 30g m^{-2}) was applied to plots with the added organic material, because the application of high carbon-low nitrogen organic wastes generally leads to a reduction in available soil nitrogen following the decomposition of the material (Wild, 1988). Cation exchange capacity analysis of samples from the plots was tested by The Scottish Agricultural College, Ayr, prior to sowing. The results were as follows: (mequ. of CEC/100g air-dry soil): dredgings, 11.26; dredgings + straw, 12.64; dredgings + spent mushroom compost, 13.78.

3.3.4 Growth of lettuce and barley on river dredgings

Both lettuce and barley were grown on outdoor plots of River Tyne dredgings. They were chosen for ease of growth and sampling and for their sensitivity to heavy metals in the soil (Davis, 1981). In view of the high tolerance to heavy metals shown by the rye-grass experiments, lettuce and barley leaves as well as the soils were analysed for metal content. Roots were not used as a measure of metal uptake as most of the metals are bound in the cell walls or outside the roots where they are not biologically active and from where they cannot be transported to the aerial parts of the plant. Pb in particular, appears to accumulate in the roots while the content in the leaves remain at a normal level until the

roots are saturated (John & Van Laerhoven, 1972).

Barley and lettuce planted on the river dredgings and arable plots emerged well, with a emergence rate for barley of between 92-96% (Table 12). The straw was not completely degraded in the soil at the time of sowing; in such plots there was a depression of the growth rate and a significant reduction ($p < 0.05$) in the yield of barley and lettuce when harvested in August 1988 (Figs. 21, 22 & 23). Growth rate of lettuce grown in the dredgings over the first 5 weeks was significantly slower ($p < 0.05$) than growth rate of lettuce on arable soil over the same period (Fig. 21), although by the time of harvesting (10 weeks), the mean yield of lettuce grown in the control soil was not significantly better than yield of lettuce grown on the dredgings (excluding dredgings+straw). Lettuce grown in the dredgings appeared healthy, and it was decided to concentrate on barley as a bioassay indicator.

In August 1988, the growth rate, root length and the yield of barley grown on plots of control soil was significantly better ($p < 0.05$) than on the dredging with either of the amendments (Fig. 22, 23, Photographs 6 & 7). Growth rate and yield of barley grown in the dredgings alone was not significantly different compared with barley grown on the dredgings+spent mushroom compost.

The metal content of the control soil lay well within the widely-accepted normal ranges for these elements (Baker & Chesin, 1975) that is, Zn at 17mg/kg (±1.1); Cd at 0.2mg/kg (±0.02); Cu at 7.0mg/kg (±0.08) and Pb at 9.2mg/kg (±0.5) (Table 13). The metal content in the River Tyne dredgings was similar to the values of the original analysis of the dredged soil at its original landing site (mg/kg): Zn at 480±1.8 [cf 490]; Cd at 2.6±0.2 [cf 2.6]; Cu at 52.2±2.0 [cf 58] and Pb at 212±13.8 [cf 210] (Fig. 24 and 25). These values were considerably in excess of levels found in the control soil.

Concentration of metals in the leaves of barley grown on the control soil were all lower than normally-accepted concentrations in plant tissue (μg/g): Zn at 2.0±0; Cd 0.01±0; Cu 2.5±0.1 and Pb 0.05±0 (Table 14).

Table 12. Rates of emergence of spring barley grown on River Tyne dredgings

Spring barley was planted in plots treated with various organic amendments in May 1988. The percentage emergence of the plants was determined for each set of five test rows and the mean value calculated.

Substrate and organic amendment	% emergence (mean)
Control (arable soil)	92
Dredgings alone	93
Dredgings + straw	96
Dredgings + spent mushroom compost	94

Analysis of data using ANOVA showed that there was no significant difference between the germination rates of River Tyne dredgings with or without amendments and the arable control.

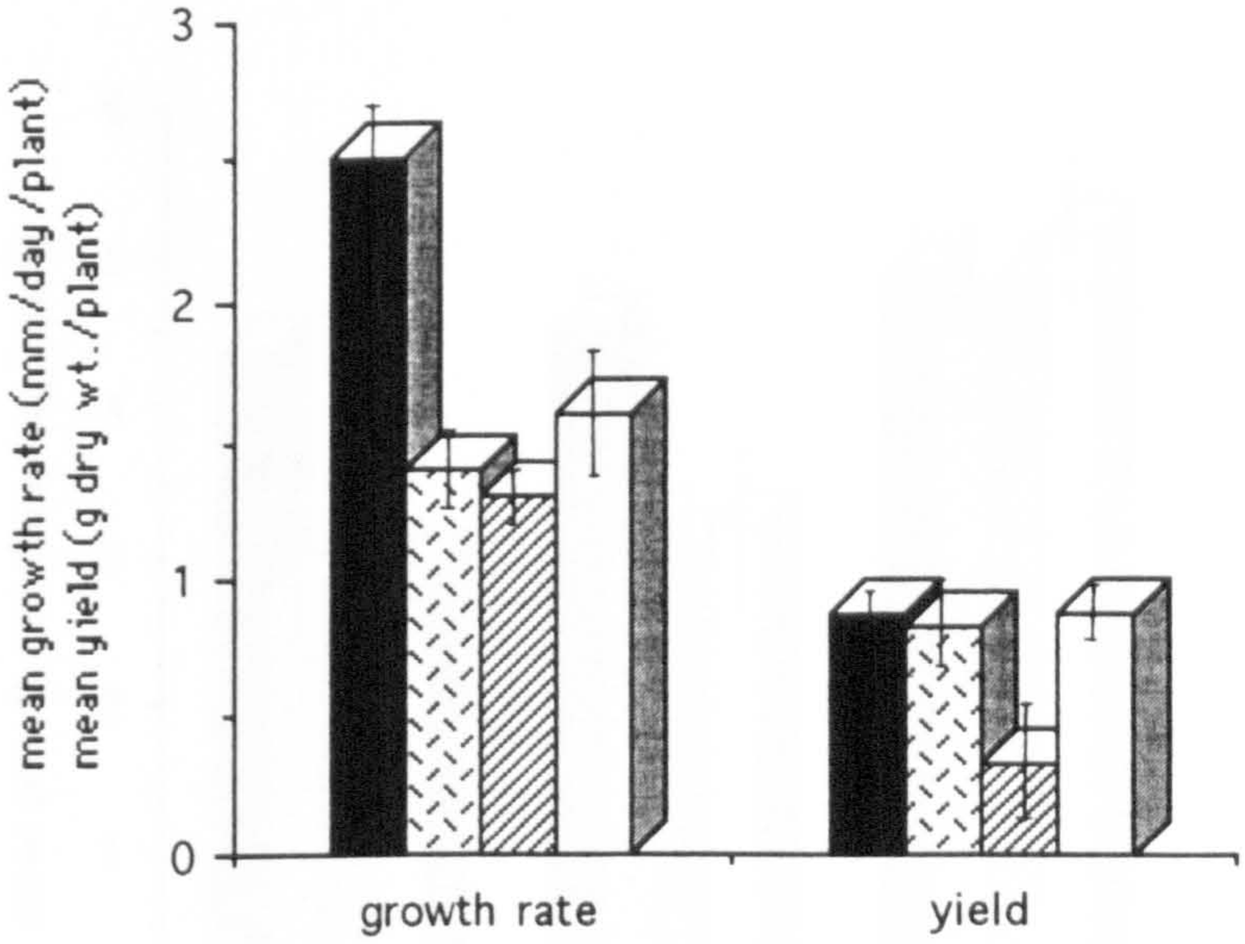


Figure 21. Growth rate and yield of lettuce grown in control soil (arable) and River Tyne dredgings.

Lettuce (*Lactuca sativa* L. var. Tom Thumb) was grown in trial plots (0.5m x 1m) outdoors in River Tyne dredgings and control soil (arable); control soil ■; Tyne dredgings ◱; Tyne dredgings + straw ▨; and Tyne dredgings + spent mushroom compost ❒. The mean growth rate was determined by measuring the diameter of plants 7, 14 and 21 days after thining at 14 days. Plants were harvested at 10 weeks when mean dry weight (exc. roots) was recorded. Error bars indicate standard deviations.

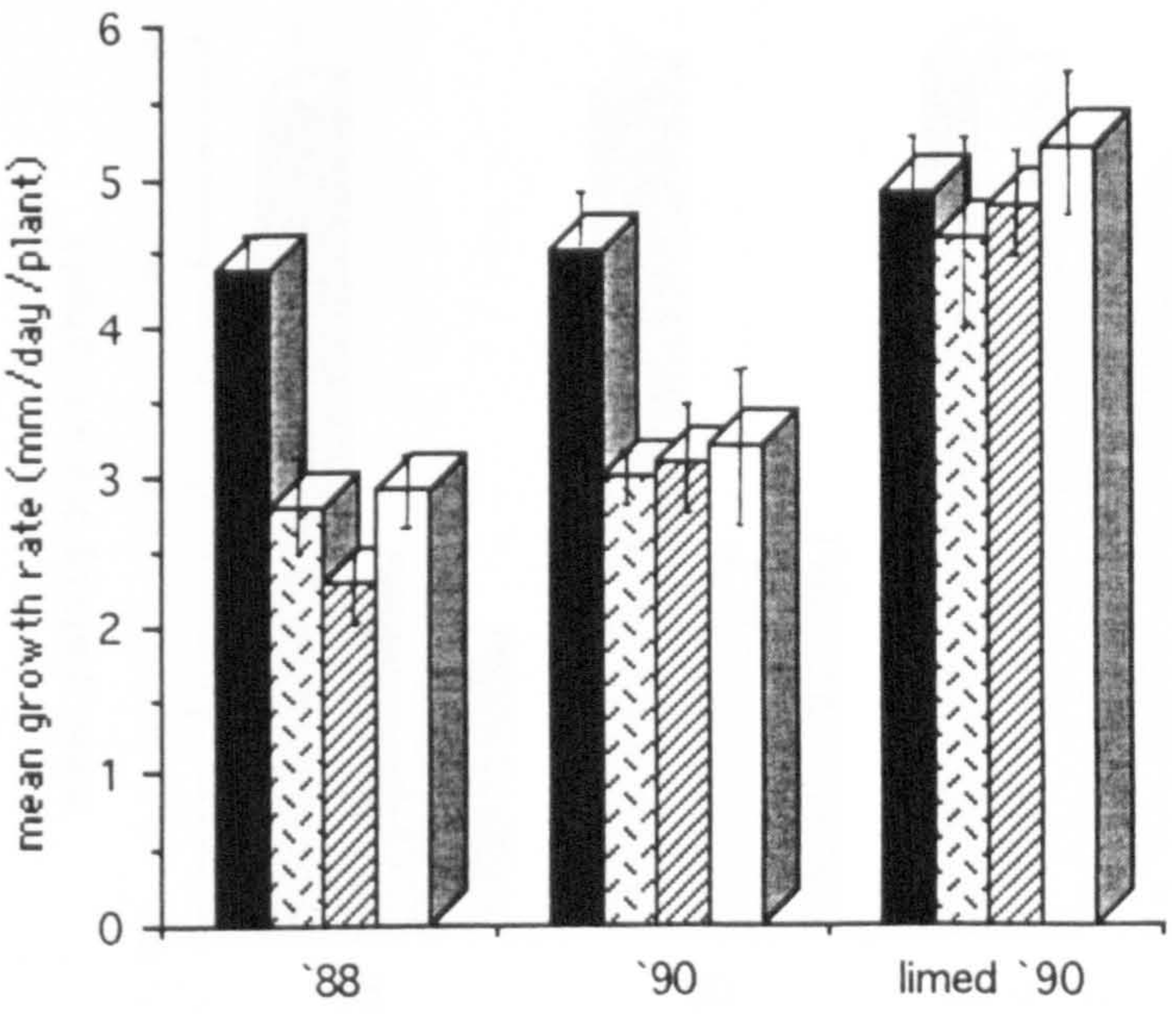


Figure 22. Growth rate of barley grown on control soil (arable) and River Tyne dredgings in 1988 and 1990.

Spring barley (*Hordeum vulgare*) was grown in trial plots (0.5m x 1m) outdoors in control soil (arable) and River Tyne dredgings; control soil ■; Tyne dredgings ▨; Tyne dredgings + straw ▨; and Tyne dredgings + spent mushroom compost ❒. The mean growth rate was determined by measuring the extension of the first leaf of each plant at 10, 17 and 24 days after seed germination. Error bars indicate standard deviations.

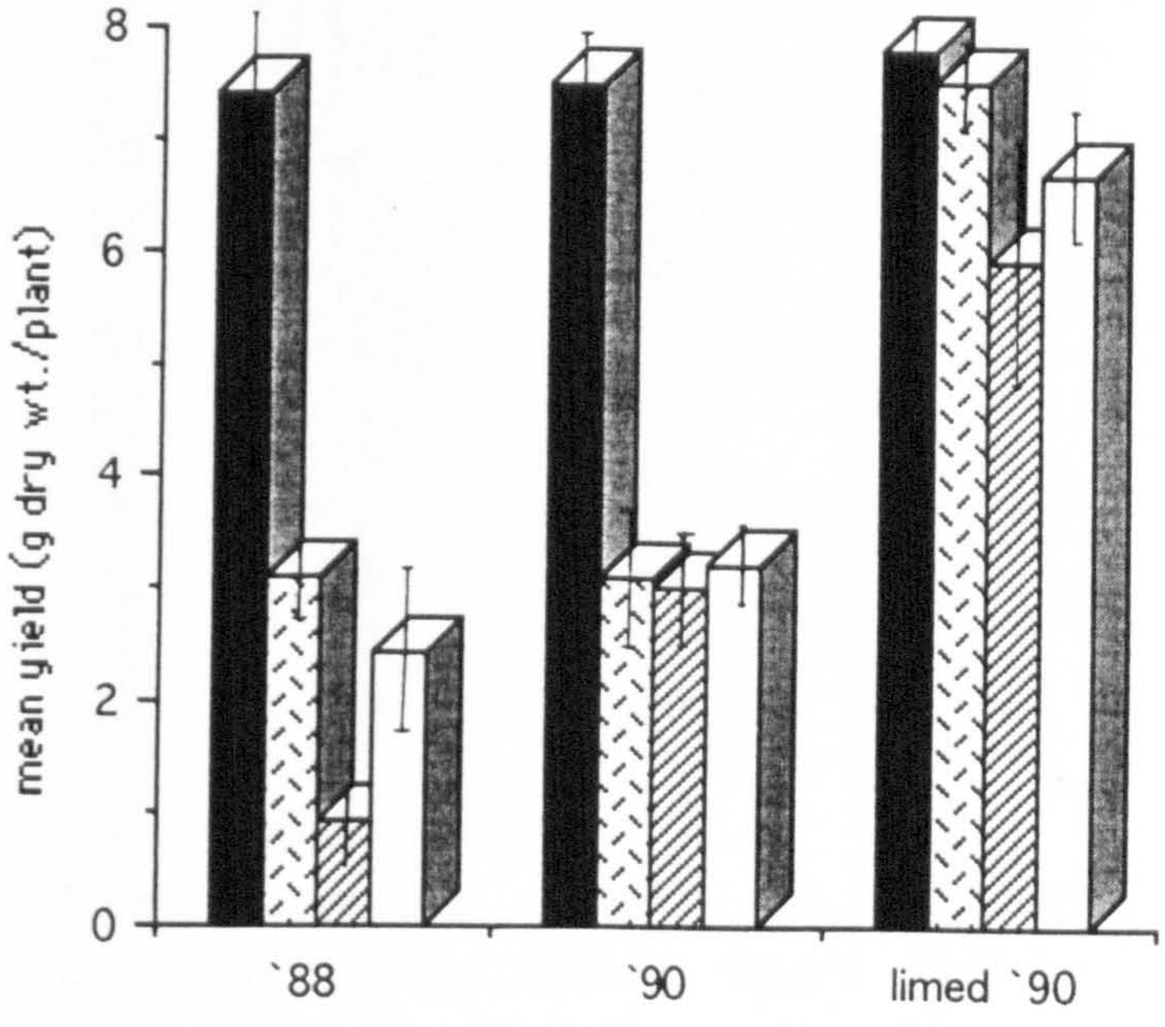
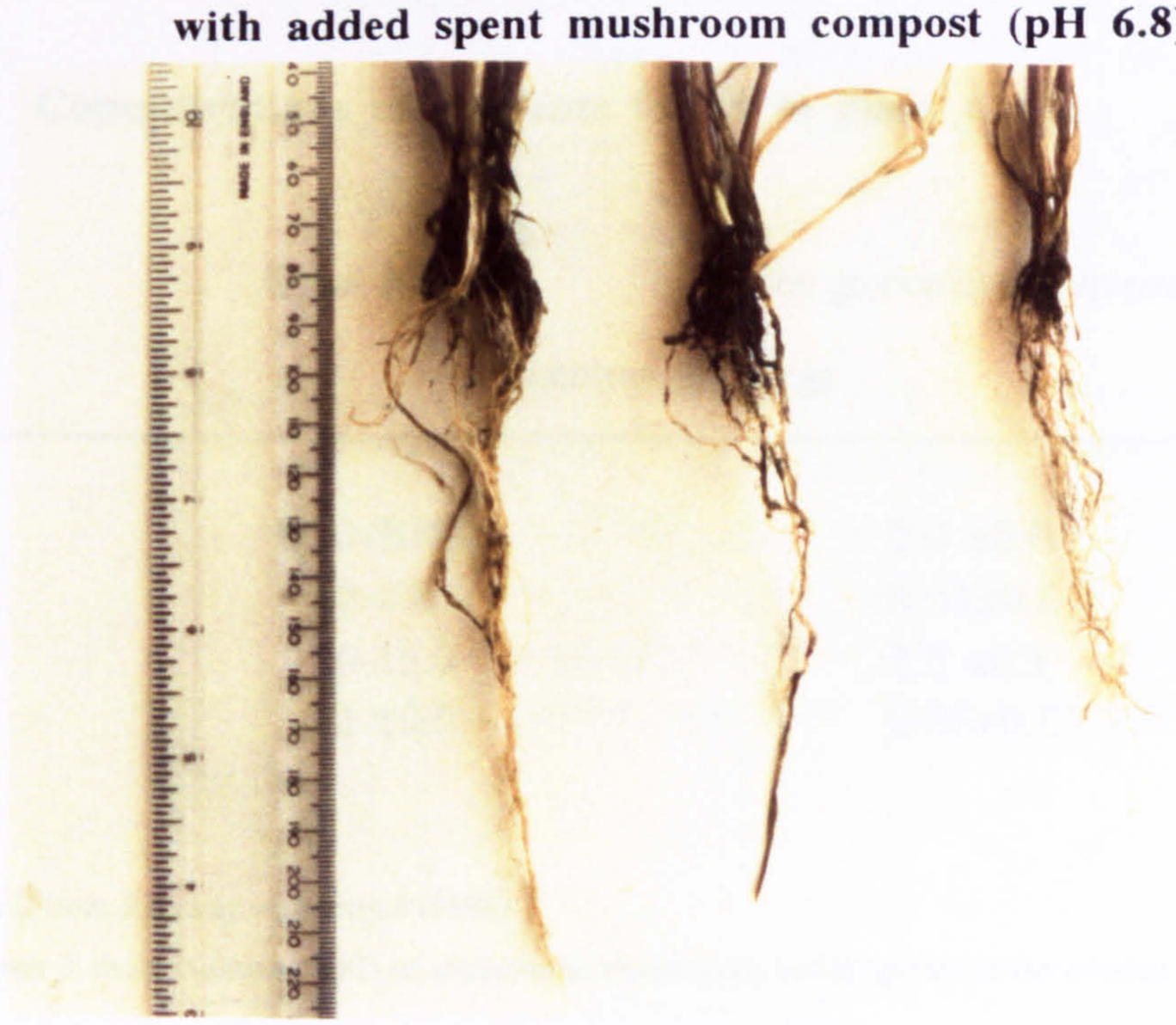


Figure 23. Yield of barley grown on control soil (arable) and Tyne dredgings in 1988 and 1990.

Spring barley (*Hordeum vulgare*) was grown in trial plots (0.5m x 1m) outdoors in control soil (arable) and River Tyne dredgings; control soil ■; Tyne dredgings ▨; Tyne dredgings + straw ▨; and Tyne dredgings + spent mushroom compost ▢. Plants were harvested at maturity (approx. 3m after sowing) and mean dry weight exc. roots was determined. Error bars indicate standard deviations.

Photograph 6. **Barley shoots grown on (L to R), arable soil, dredgings with added straw (pH 6.7), dredgings with added spent mushroom compost (pH 6.8).**



Photograph 7. **Close up of barley shoots grown on (L to R), arable soil, dredgings with added spent mushroom compost (pH 6.8), dredgings with added straw (6.7).**

Table 13. Element variations in soils

Element	Usual Range	Average	In arable soil in present plant growth experiment
	concentration (mg/kg)		
Zn	10-300	50.0	17.0± 1.1
Cd	0.01-0.70	0.5	0.2± 0.02
Cu	2-100	20.0	7.0± 0.08
Pb	2-200	10.0	9.2± 0.5

Data in column 1,2, and 3 from Baker & Chesin (1975)

Figures in column 4 list analytical average of triplicate samples ±SD

Table 14. Concentrations of elements found in plant tissue

Element	Usual Range	Barley grown in arable soil
	concentration (μg/g)	
Zn	15.0-200.0	2.0 ±0.0
Cd	0.2-0.8	0.01±0.0
Cu	4.0-15.0	2.5 ±0.1
Pb	0.1-10.0	0.05±0.0

Data in column 2 from Alloway & Morgan (1986)

Figures in column 3 show the mean ±SD of triplicate analyses from barley grown in the present experiments.

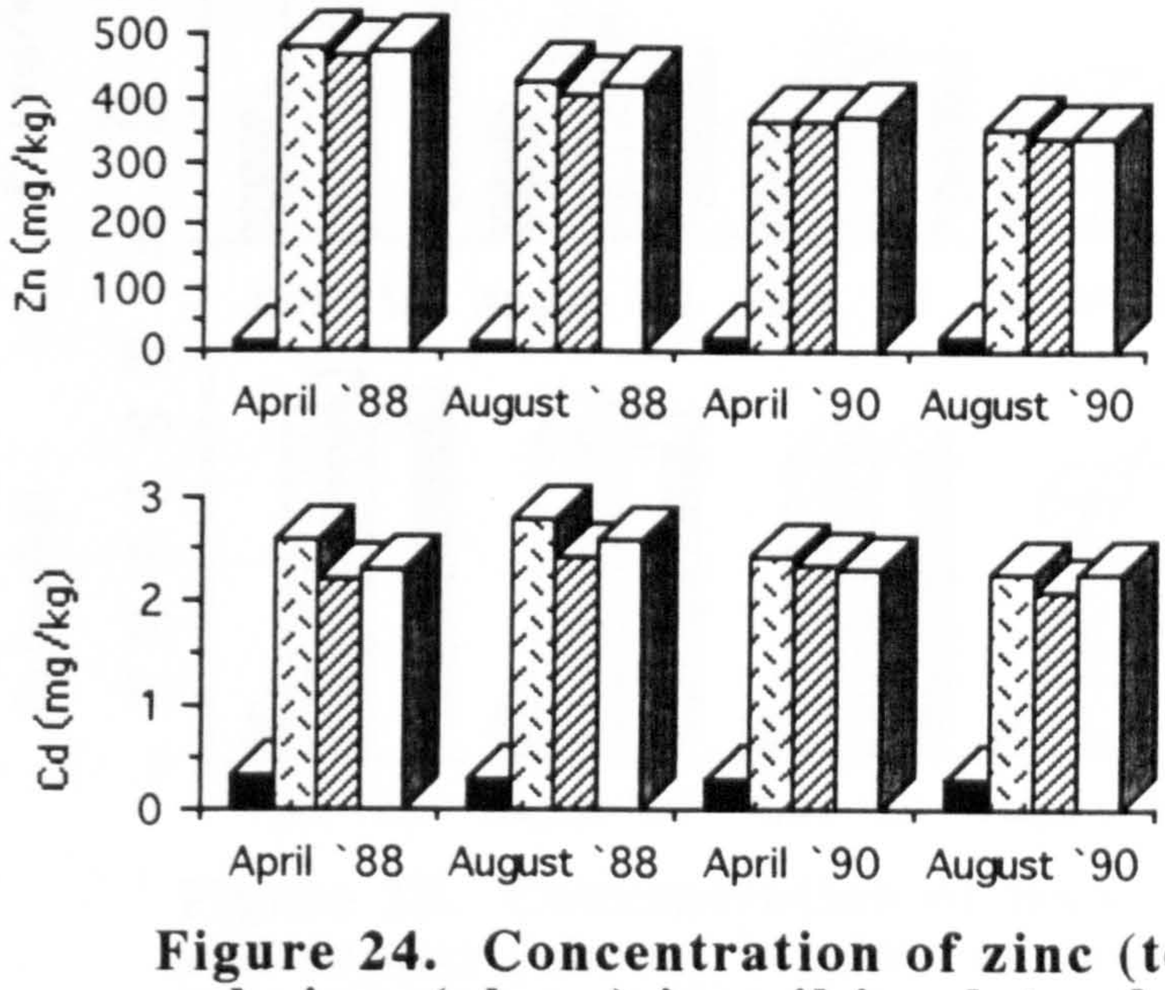


Figure 24. Concentration of zinc (top) and cadmium (above) in soil in plots of control soil (arable) and River Tyne dredgings.

Concentrations of zinc and cadmium in plots (0.5m x 1m) of control soil (arable) and River Tyne dredgings; control soil ■; Tyne dredgings ▱; Tyne dredgings + straw ▨; and Tyne dredgings + spent mushroom compost ❒. Concentrations of zinc (top) and cadmium (above) in soil during April 1988, August 1988, limed April 1990 and limed August 1990 were EDTA (0.5M) extracted followed by atomic absorption spectroscopy.

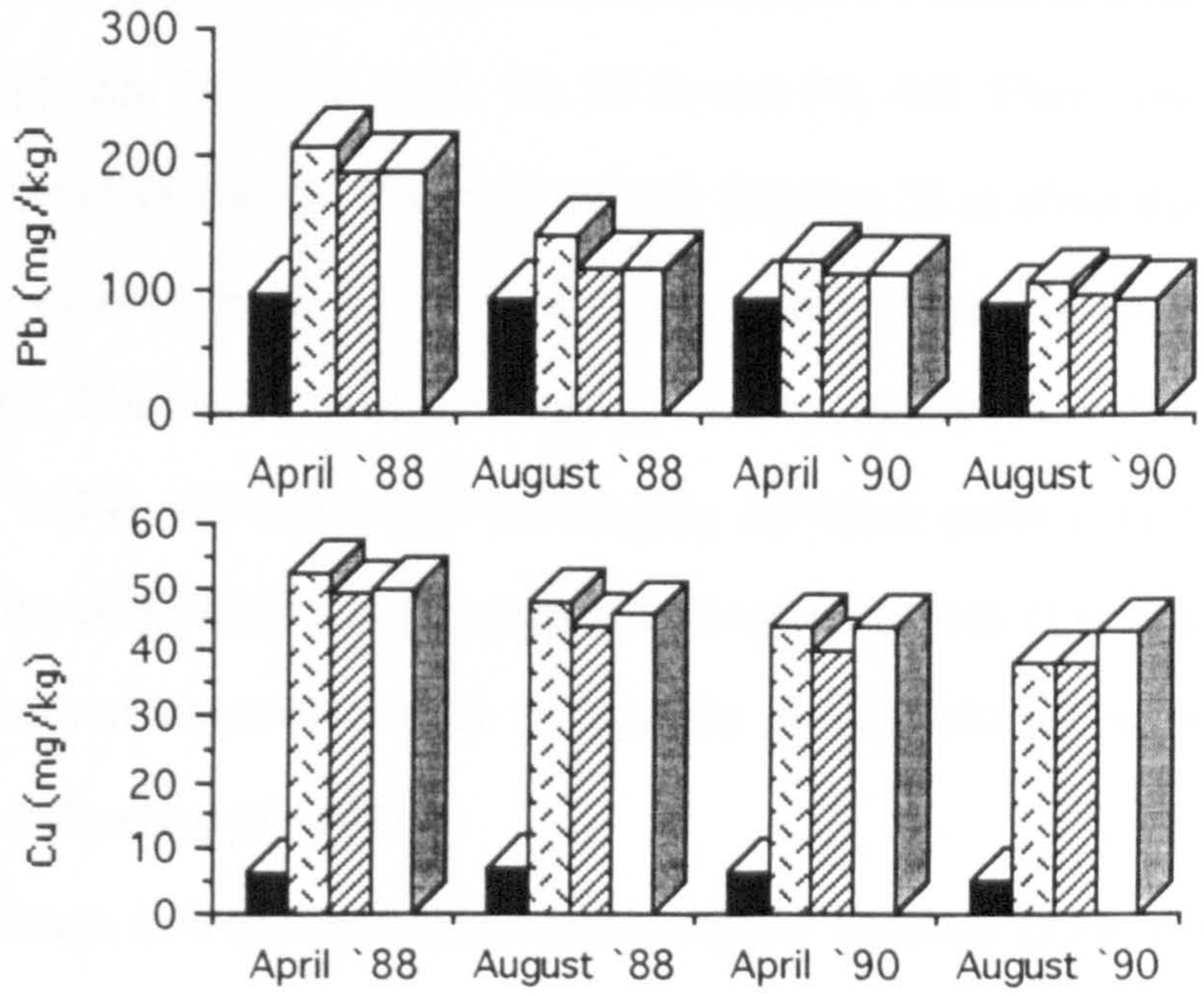


Figure 25. Concentration of lead (top) and copper (above) in plots of control soil (arable) and River Tyne dredgings.

Concentrations of lead and copper in plots (0.5m x 1m) of control soil (arable) and River Tyne dredgings; control soil ■; Tyne dredgings ◪; Tyne dredgings + straw ▨; and Tyne dredgings + spent mushroom compost ❒. Concentrations of lead (top) and copper (above) in soil during April 1988, August 1988, limed April 1990 and limed August 1990 were EDTA (0.5M) extracted followed by atomic absorption spectroscopy.

Plant tissue analysis of leaves of barley grown on river dredgings indicated the mean concentration of metals were as follows (μg/g): Zn 242±13.6; Cd 0.6±0.02; Cu 25±1.4 and Pb 4.0±0.01 (Figs. 26, 27). Samples of oven-dried barley were also sent to Agricultural Development & Advisory Service (ADAS) for metal analysis. They reported levels of (μg/g): Zn, 260; Cd, 0.63; Cu, 27.5; and Pb, 4.5. They also determined the titanium (Ti) level in barley. The ratio of soil Ti/plant Ti is often used to assess soil contamination of plant material as there is a high soil/plant ratio of around 10,000:1 (Mitchell, 1960). The low Ti level (1.22mg/kg) found in barley fell within the 0.33-3.3 for kale (Bowen, 1974) and within the 0.2-5mg/kg for clean pasture herbages (Mitchell, 1960), indicating that barley was not unduly contaminated with Ti and therefore soil. Zn and Cu were both at concentrations in the actively growing tissues of the plant at which yield is reduced (Davis *et al.*, 1978).

At this stage, as it appeared unlikely that organic additions alone would be sufficient to mitigate the effects of the metals, it was decided to raise the pH of the dredgings to between 7.1 and 7.3 though over-liming was to be avoided as it could induce deficiency in manganese and boron (MAFF, 1988). When lime was added to raise the pH of the river dredgings in the plots to above pH 7.1, the growth rate of the barley in all the plots improved, particularly that in the dredging+spent mushroom compost (Fig. 22) when it was compared with unlimed and 1988 rates. Yield of barley (whole plant excluding roots) grown in the dredgings improved significantly from the mean of 2.4±0.5 dry wt. per plant (excluding dredgings+straw) in 1988 to 6.8±0.7 dry wt. per plant in 1990. (Photographs, 8, 9 & 10). Of the river dredgings, yield was highest in dredged soil without any amendments (Fig. 23). ANOVA demonstrated no statistically significant difference between the mean yields and growth rate in 1990 of barley grown on arable soil and of that grown in any of the plots of limed river dredgings. When the mean yields in 1988 and 1990 (limed) were compared there was a statistically significant difference ($p < 0.05$) between all plots of dredgings (Fig. 23).

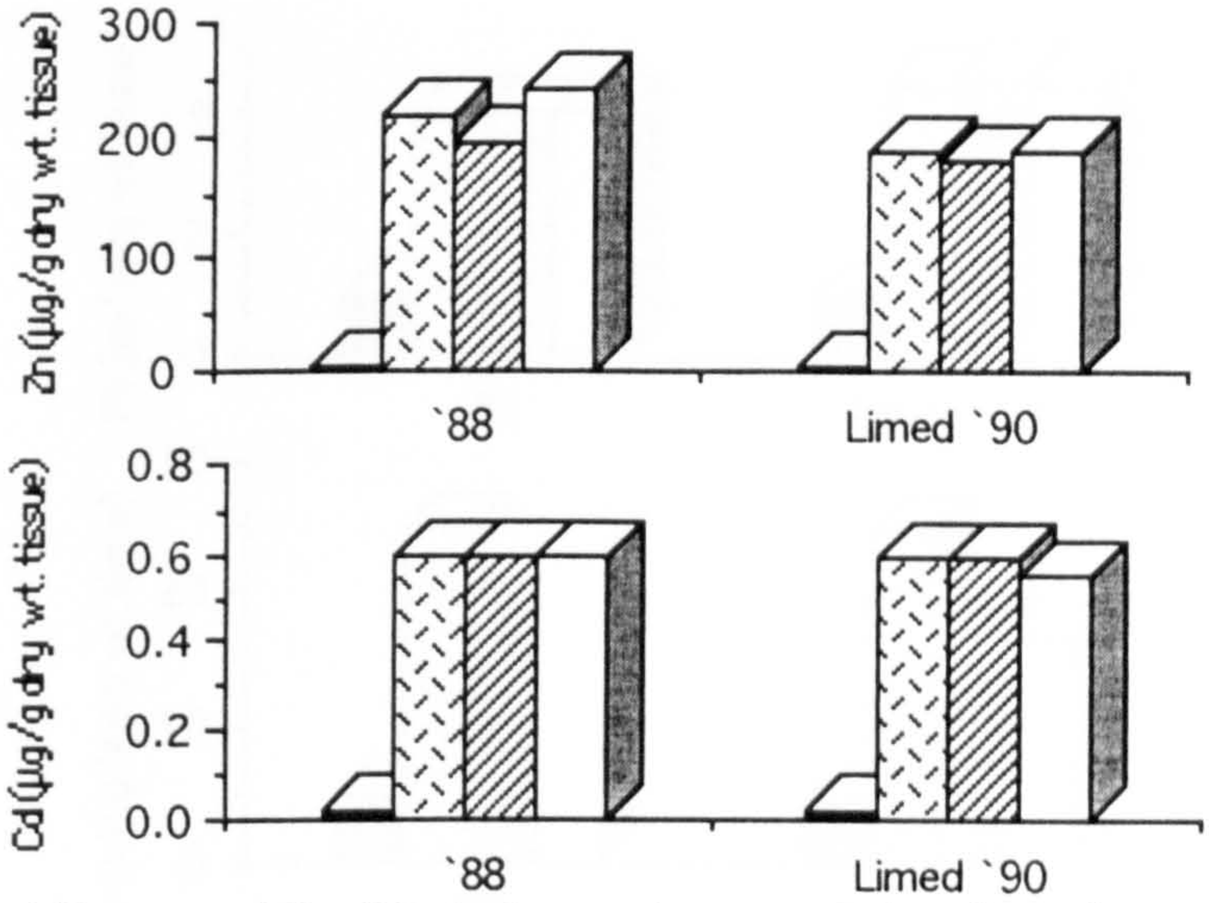


Figure 26. Metal content of barley leaves, zinc (top), cadmium (above), grown in control soil (arable) and River Tyne dredgings.

Spring barley (*Hordeum vulgare*) was grown in trial plots (0.5m x 1m) outdoors in plots of control soil (arable) and River Tyne dredgings; control soil ■; Tyne dredgings ▨; Tyne dredgings + straw ▨; and Tyne dredgings + spent mushroom compost ❒. Following harvest in 1988 and 1990, barley leaves were acid digested (Section 2.4) and zinc (top) and cadmium (above) levels were determined by atomic absorption spectroscopy.

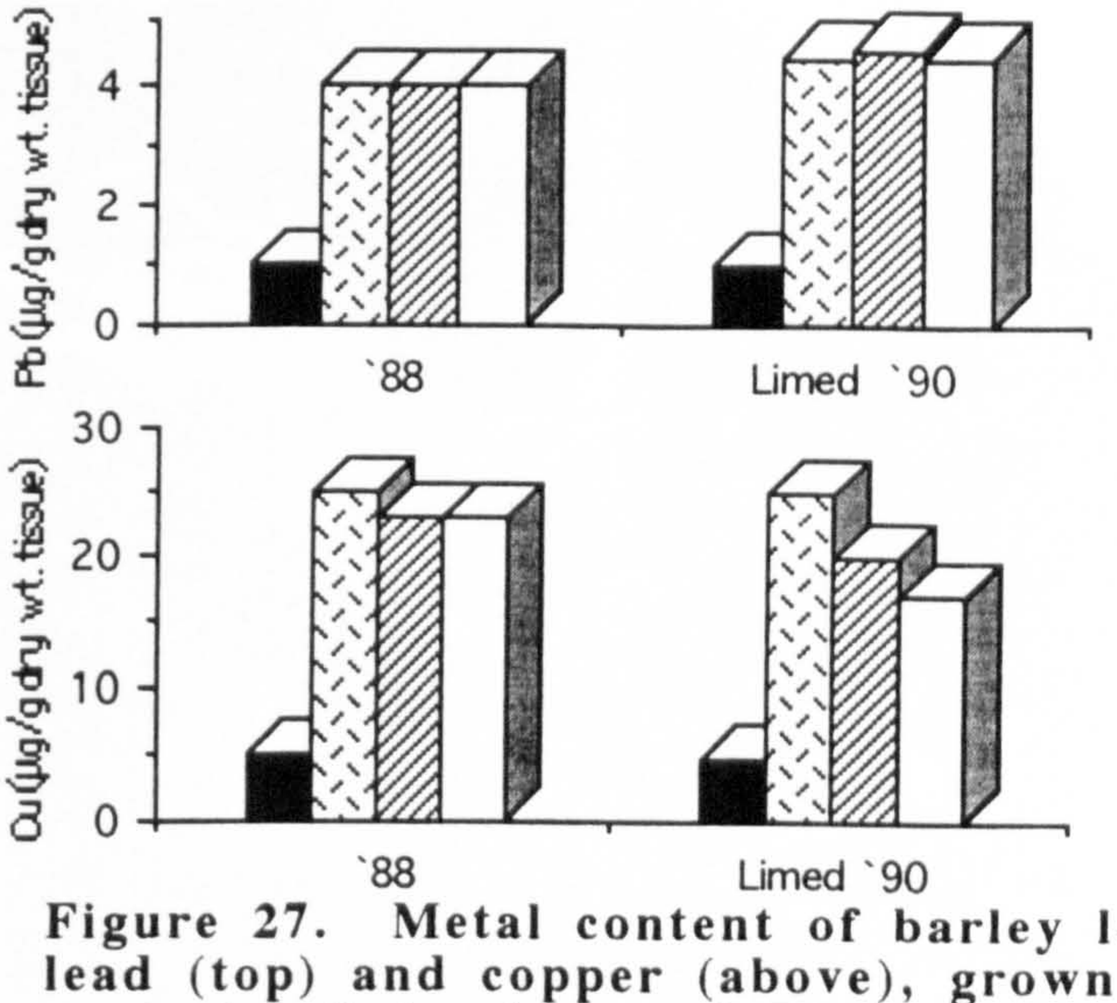
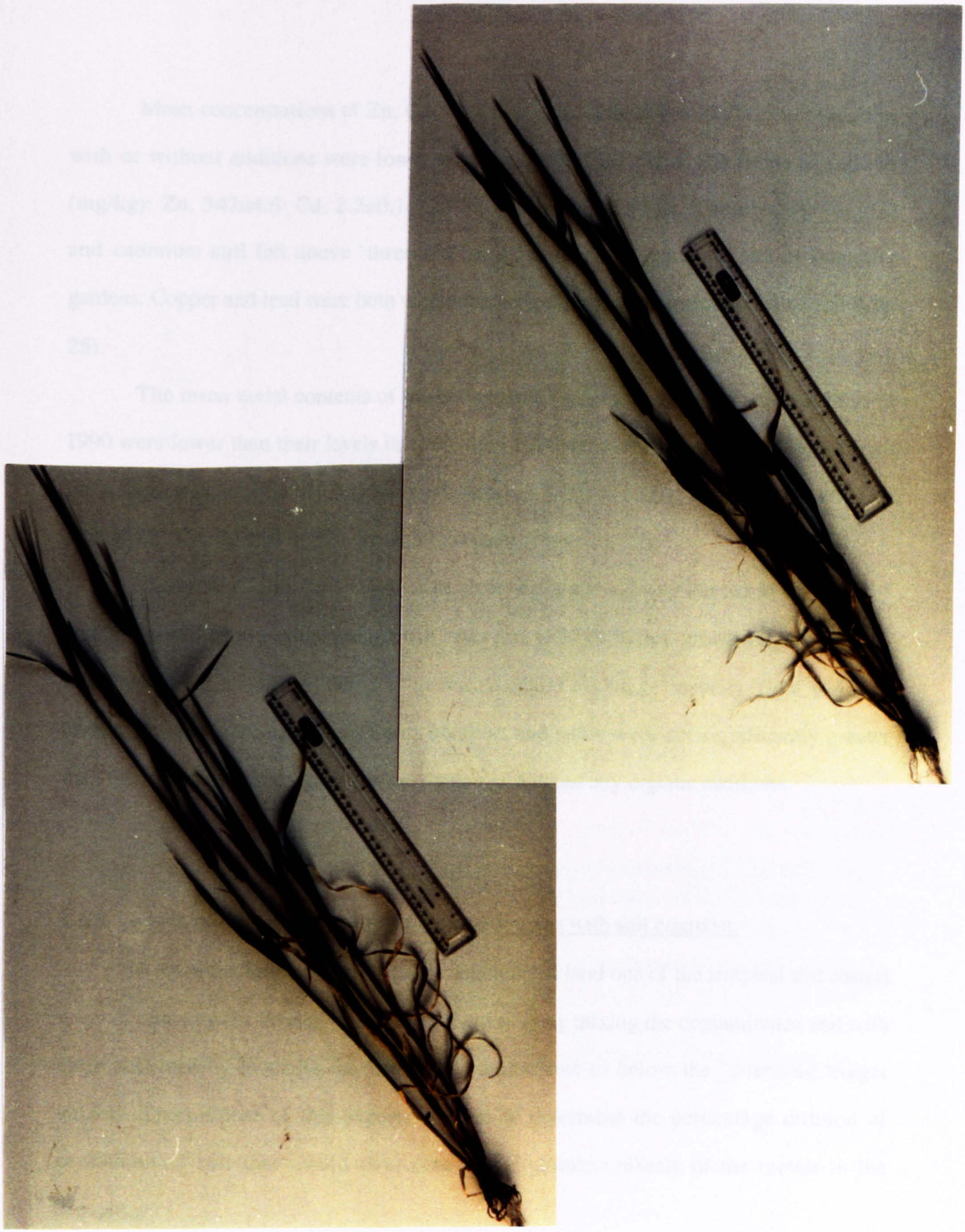


Figure 27. Metal content of barley leaves, lead (top) and copper (above), grown in control soil (arable) and River Tyne dredgings.

Spring barley (*Hordeum vulgare*) was grown in trial plots (0.5m x 1m) outdoors in plots of control soil (arable) and River Tyne dredgings; control soil ■; Tyne dredgings ▨; Tyne dredgings + straw ▨; and Tyne dredgings + spent mushroom compost ❐. Following harvest in 1988 and 1990, barley leaves were acid digested (Section 2.4) and lead (top) and copper (above) levels were determined by atomic absorption spectroscopy.

<arable soil> / <dredgings>

Photograph 8. **(L to R) Barley growing on arable soil (pH 6.4) and dredgings (pH 7.2).**



Photographs 9 and 10. (L to R) Growth of barley on arable soil (pH 6.4) and dredgings (pH 7.2).

Mean concentrations of Zn, Cd, Cu and Pb available in the 1990 limed dredgings with or without additions were lower than the 1988 (Figs. 24 & 25) levels as follows (mg/kg): Zn, 347±4.6; Cd, 2.2±0.1; Cu, 39.7±2.4; Pb, 97.5±4.1. These values for zinc and cadmium still fall above `threshold trigger values` for metals in soil of domestic gardens. Copper and lead were both within the normal ranges for uncontaminated soil (Fig. 25).

The mean metal contents of the barley leaves grown in all the limed dredgings in 1990 were lower than their levels in 1988, (and 1990 unlimed, not illustrated) apart from Pb which was marginally higher, and were as follows ($\mu g.g^{-1}$): Zn, 187±4.6; Cd, 0.58±0.02; Cu, 20.6±0.9; Pb, 4.6±0.5 (cf4.0±0) (Figs. 26, 27).

Therefore adding lime to the Tyne river dredgings to bring the pH to between 7.1 and 7.3, significantly improved growth rates and yield of barley grown on it. Although straw and spent mushroom compost increased cation exchange capacity, final yields of barley grown in both spent mushroom compost and straw were not significantly greater than final yields of barley grown in the dredgings without any organic additions.

3.3.5 Growth of barley on mixtures of Tyne dredgings with soil compost.

In the remediation of industrially contaminated land one of the simplest and easiest ways of reducing the level of contaminants in soil is by mixing the contaminated soil with clean soil thereby reducing the levels of contaminants to below the `threshold trigger values`. The purpose of this experiment was to determine the percentage dilution of contaminated soil that would overcome the phytotoxic effects of the metals in the dredgings.

The growth rate and yield for barley grown in mixtures consisting of dredgings with John Innes No. 1 compost are shown in Fig. 28. Growth rate was only marginally affected for barley even with the higher percentages of dredgings (Fig. 28) but addition of

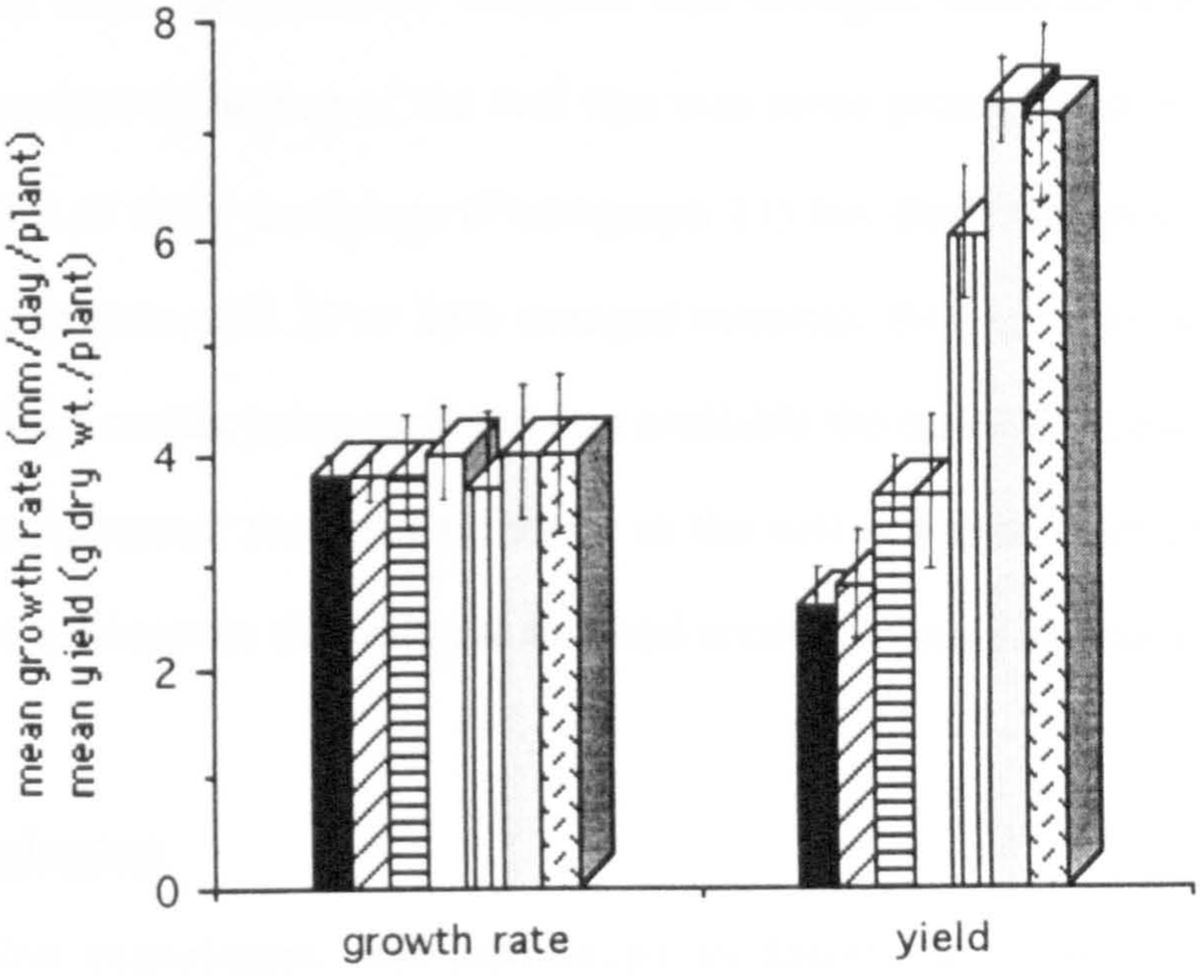


Figure 28. Growth rate and yield of barley grown in mixtures of River Tyne dredgings and soil compost.

Spring barley (*Hordeum vulgare*) was grown under glass in pots (15 diam.) in mixtures of River Tyne dredgings and control (soil compost) in the following percentage of dredgings: 100 ■; 75 ▨; 66 ▤; 50 ❒; 33 ▥; 25 ⬚; 0 ◪. The mean growth rate was determined by measuring the extension of the first leaf of each plant 10, 17 and 24 days after seed germination. Plants were harvested at maturity (approx. 3m after sowing) and mean dry weight exc. roots determined. Error bars indicate standard deviations.

50% (w/w) or more dredged material depressed the growth yield by more than 50% (Fig. 28). Analysis of metal concentrations in pots showed the expected decrease as the proportion of soil compost increased in the mixtures (Table 15). Analysis of the plants after growth in these mixtures of compost and dredged material are shown in Table 16. Yellowing and withering of the leaf tips was more pronounced in pots with the greatest percentages of river dredgings (Photograph 11) but there was practically no withering or yellowing in pots with 33 or 25% dredged material. Simple dilution effects, together with the dredgings metals being rendered less available through cation exchange and complexing with organic matter and clay minerals in the soil compost fraction of the mixtures was thought to account for the decrease in metal content of soils and barley.

3.3.6 Replanting

This experiment was introduced to determine if the larger root surface area produced in arable soil or soil compost would be affected by metal toxicity when compost-raised plants were transplanted to dredging mixes, and be reflected in plant yields. The root length and yield of barley was measured in a) barley grown on soil compost for 4 weeks and replanted in dredgings for 8 weeks and b) barley grown on dredgings for 4 weeks and replanted into soil compost for the subsequent 8 weeks. Controls where plants were transferred into same medium and undisturbed controls were included.

Root length was severely inhibited when plants were grown in the river dredgings (Photographs 12 & 13) (Fig. 29). Barley grown in dredgings had a mean root length of 12cm compared with a mean root length of 44cm when grown in soil compost. Yield of barley initially grown in the dredgings and transferred to proprietary soil compost (4.2g dry wt./plant) was higher than comparable pots of barley grown on river dredgings and not transferred (2.9g dry wt./plant) (not illustrated). Barley grown on soil compost and transferred to dredgings had the lowest yield (1.8g dry wt./plant) (Fig 29). Statistical analysis confirmed mean yield of barley grown on soil compost and transferred to river

Table 15. Analysis of metals in mixtures of River Tyne dredgings and soil compost.

Dredged material in soil mixture (%)	Concentration of metal in soil mixtures (mg/kg)			
	Pb	Cu	Cd	Zn
100	103	50	2.8	430
75	85	44	2.4	370
66	73	36	2.2	245
50	54	30	1.5	245
33	49	24	1.2	133
25	39	18	0.9	94
0	9	5	0.1	27

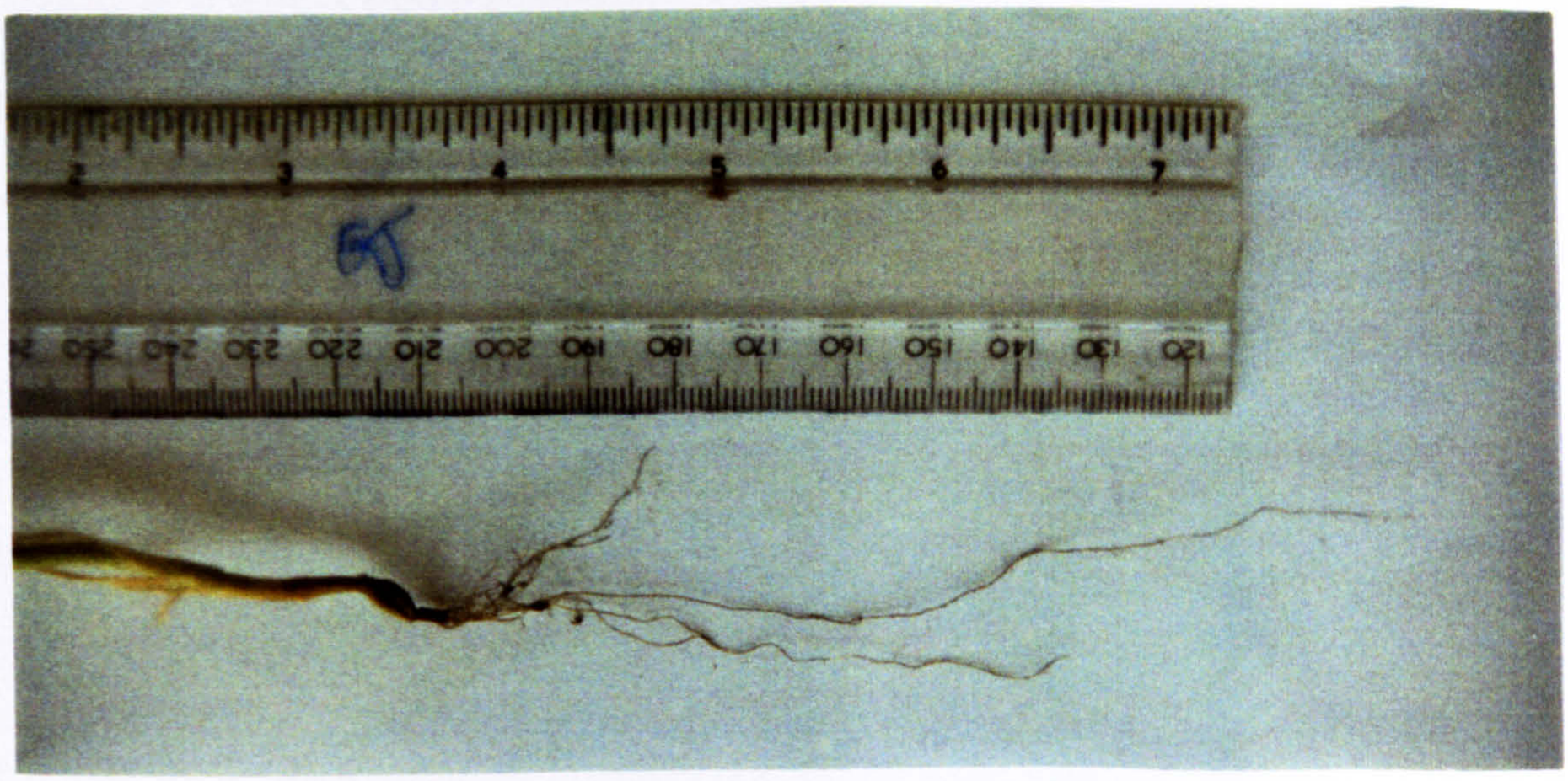
Standard Deviation under 7%

Table 16. Analysis of metals in barley grown in mixtures of river Tyne dredgings and soil compost

Dredged material in soil mixture (%)	Concentration of metal in barley leaves (μg/g)			
	Pb	Cu	Cd	Zn
100	2.0	25.0	0.30	140
75	2.0	26.0	0.27	132
66	2.0	24.0	0.30	96
50	1.8	24.0	0.25	62
33	2.0	20.5	0.20	50
25	2.0	14.0	0.20	40
0	0.1	2.0	0.03	19

Standard Deviation under 7%

Photograph 11. **Barley grown on (L to R), control (soil compost) (pH 6.5), 50/50 soil compost: dredgings mix (pH 6.6), dredgings (pH 6.7).**



Photograph 12 and 13. Close up of barley roots at 2 weeks grown in control (soil compost) soil (top) and dredgings (pH 6.7) (above).

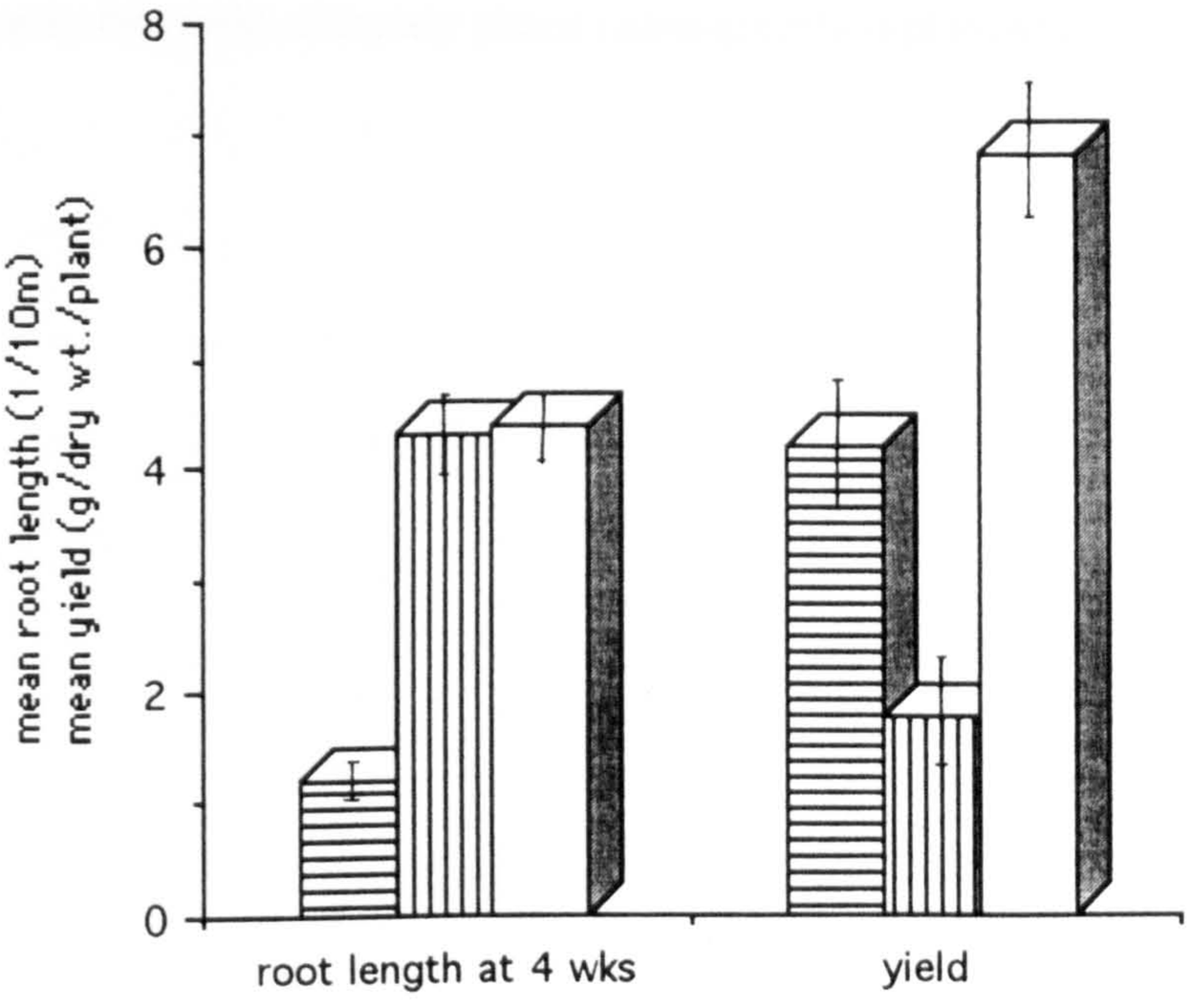


Figure 29. Root length and yield of barley grown on control (soil compost) or River Tyne dredgings.

Spring barley (*Hordeum vulgare*) was grown in; soil compost ▥; soil compost ❐; or River Tyne dredgings ▤ and uplifted at 4 weeks when root length was measured. The plants were then transferred from: soil compost to dredgings ▥; from soil compost to soil compost ❐; from dredgings to soil compost ▤. Values are means of three replicates. Error bars indicate standard deviations.

dredgings was significantly poorer than barley grown on soil compost alone ($p < 0.05$) or on dredgings and transferred to soil compost ($p<0.05$). In conclusion, these results strongly indicate that the larger root surface area of compost-raised plants caused a severe reduction in the yield of barley plants subsequently replanted in the dredgings.

4.0 Experiments with soil from the Derwenthaugh site

4.1 Derwenthaugh site history

The Derwenthaugh coking plant was built in 1929 by the Consett Iron Co. and consisted of 2 batteries of 28 ovens each with associated by-product, steam raising and power generating plants. A third battery of 10 ovens was added in 1930 and a further 20 ovens were built in 1936 giving a final capacity of 475,000 tonnes of coal per annum. The plant originally produced blast furnace, domestic and industrial coke but was changed over to the production of foundry coke in 1960. By-products recovered included tar, sulphate of ammonia, crude benzole and town gas (Ogilvie, 1978). The whole plant was closed in 1985. In 1989 Gateshead Metropolitan Council had acquired the site and were investigating methods of remediating the site with a view to its redevelopment and the building of a leisure complex. The Derwenthaugh site was selected for study because, in contrast to be the Tyne dredgings, it was thought to contain high levels of phenolic contamination (H. Shipley, Gateshead Borough Metropolitan Council, Planning Dept. pers. comm., 1989).

4.2 Analysis of Derwenthaugh soil

Initial inspection of the site showed large areas were in a derelict condition with the coking ovens partly demolished. Evidence of contamination was visible e.g. `Blue Billy`, noxious odour, dark discolouration of soil on parts of the site. An area devoid of vegetation (5m x5m) was selected for sampling.

Analysis of Derwenthaugh soil found levels of phenol and coal tars compounds considerably in excess of the DOE`s (1987) guidelines for contaminants associated with former coal carbonisation sites to be used in landscaped areas (Table 17). Other contaminants were below the `threshold trigger values`. Total and available heavy metals (Table 18) were also below `threshold trigger values`; metal analyses done

Table 17. Analysis of Derwenthaugh soil.

Contaminant	Conc. of contaminant (mg/kg soil dry wt.)*	Threshold trigger values**
pH	5.5	5
Phenol	160.0+	5
Polyaromatic hydrocarbons	190,000=	50
Cyanide (free)	5.0	25
Thiocyanate	14.5	50
Sulphate	53.0	2000
Sulphide	3.0	250

* Values were the mean of at least three determinations

** Threshold trigger concentrations (mg/kg air-dried soil) for contaminants associated with former coal carbonisation sites for use in play and landscaped areas (DOE, 1987)

= Although this figure includes appreciable amounts of aliphatic hydrocarbons, heterocycles and phenolics etc., given the high overall concentration, the PAH concentration can be expected to exceed the threshold value.

+ Analysis had shown (p92) phenolic compounds included o-cresol, resorcinol, 3,4-dimethylphenol and 2,4,6-trimethylphenol.

Table 18. Metal analyses of Derwenthaugh soil

Element *	Concentration of metal ion in soil (mg/kg)=	
	Arable soil[v]	Derwenthaugh soil
	pH 7.13	pH 7.0
Zinc (available)	29.4	45.4
Zinc (total)	493.0	352.5
Cadmium (available)	0.2	0.0
Cadmium (total)	1.5	1.6
Copper (available)	30.5	7.1
Copper (total)	343.7	115.2
Lead (available)	33.7	36.0
Lead (total)	226.5	290.0
Arsenic (available)	0.1	5.1
Nickel (available)	1.2	0.6
Chromium (available)	0.1	0.3
Iron (available)	113.0	120.0
Iron (total)	19429.0	25783.0
Manganese (available)	46.8	12.2
Manganese (total)	299.7	162.7

[v] Arable soil from adjacent uncontaminated field

* Total metal analyses by W.Stelling, Geography Dept., University of Newcastle-upon-Tyne.

= Standard deviations of three values < 5% of mean.

Threshold trigger concentrations (mg/kg air-dried soil) for parkland (DOE, 1987)

Cadmium (total)	15
Lead (total)	2,000
Arsenic (total)	40
Zinc (available)	300
Copper (available)	130
Nickel (available)	70

independently on the Derwenthaugh soil by W. Stelling, Geography Dept. Newcastle University, confirmed my available metal determinations (<5%), so it was clear that metal contamination was not the problem on this site.

4.2.1 GC-MS analysis of Derwenthaugh soil

A sample of Derwenthaugh (DWH) soil was analysed by gc-ms for quantitative and qualitative determination of polyaromatic hydrocarbons to determine the extent of contamination as suggested in the ICRCL guidelines for analysis of coal carbonisation sites (DOE, 1987).

DWH soil (50g) was extracted in dichloromethane:methanol and a sample of the extract run on silica (Merck Kieselgel type 60G) t.l.c. plates. Four distinct bands corresponding to the R_f values of the standards were removed and called aliphatics (ALI) (i.e. n-alkanes); light aromatics (LARO)(i.e. mono-aromatics); aromatics and some polar compounds (HARO)(i.e. tri-aromatics); nitrogen, sulphur, oxygen, polar compounds and spot line (NSO). The separated bands were extracted in organic solvent and injected into the gc-ms for analysis. Using a highly efficient column and a data acquisition system that continuously scanned the mass spectra, spectra were recorded during the entire duration of the gas chromatogram. Processing of this data was carried out to determine all mass spectral peak heights. The raw data for each mass spectrum were summed to give the total ionization of each scan which was stored along with the scan index number. A plot of total ion intensity *vs.* scan index number revealed the gas chromatogram. In all four fractions of the DWH soil, the total ion chromatogram (Fig. 30) displayed a prominent baseline hump, indicating the presence of abundant unresolved complex material (UCM). This is a common feature of partly-degraded high molecular weight or low volatile material. T.l.c. did not fully resolve the material, some compounds being common to all fractions. The presence of this unresolved complex material and the complexity of the mixture made identification and quantification of

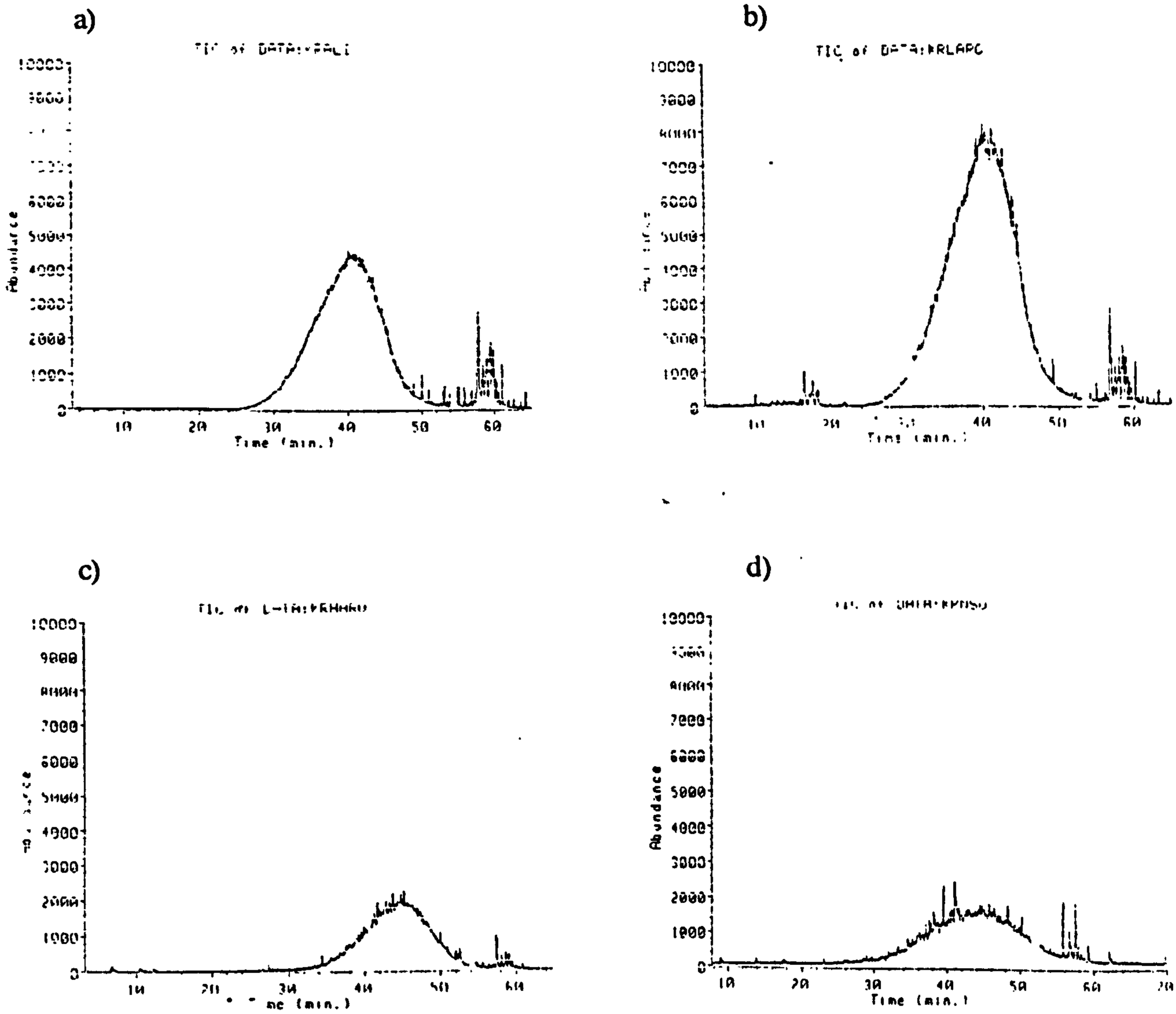


Figure 30. Total ion chromatograms of Derwenthaugh (DWH) soil.
A sample of oven-dried soil (50g) was extracted in a Soxhlet apparatus with dichloromethane:methanol 97:3 for 72h. The extract was concentrated and spotted onto a silica gel t.l.c. plate and run in dichloromethane for the first 2cm above then when dry, fully developed in light petroleum. The plate was divided into four bands corresponding to the R_f values of the standards with the less polar compounds at the foot of the plate. The bands were scraped off the plate and then the hydrocarbons were removed from the silica gel by extraction with light petroleum/dichloromethane and injected into gc-ms for analysis.
The total ion chromatogram (i.e. total ion content) of the four fractions of the DWH soil are illustrated: a) aliphatics (ALI); b) light aromatics (LARO); c) heavy aromatics (HARO) and d) nitrogen, sulphur and oxygen-containing compounds (NSO).

many of the components extremely difficult, but spectra of interest could be selected by the computer. The most usual presentation of a mass spectrum is in the form of a bar graph in which the m/z values are plotted horizontally and the abundances vertically. The most abundant ion is arbitrarily given an intensity of 100%. Identification of the unknown compound can be made by comparing its mass spectrum with reference spectra held in computer data base or by comparison with other published data (Mass Spectrometry Data Centre, 1974; Lee *et al.*, 1981). Phenanthrene (M.W. 178; retention time 36.5min in the LARO soil fraction) and dimethylnaphthalene (M.W. 166; retention time 18.0min in the HARO soil fraction; Fig. 31), were two of the polyaromatic hydrocarbons present in the soil sample (Environmental Resources Ltd., 1987) that are included in the US Environmental Protection Agency list of `priority pollutants.`

4.2.2 Infra-red analysis of Derwenthaugh soil

An i.r. scan of DWH soil, examined as a solid dispersion in a KBr disc showed a similar spectrum to that of the River Tyne dredgings, but with a much stronger intensity of hydrocarbons in the region around 3000cm^{-1}. The band at 3431 cm^{-1} was thought to indicate unsaturated hydrocarbons, while the cluster of bands just below 3000cm^{-1} was probably due to saturated hydrocarbons. The bands at 1456 and 1506cm^{-1} were thought to indicate the presence of phenolic compounds as they fall within the `phenolic fingerprint region`, though it is possible they could be due to the stretching of the methyl groups associated with the strong hydrocarbon presence (Fig. 32).

In conclusion, the DWH soil contained appreciable quantities of organic contamination in the form of phenolic compounds and polyaromatic hydrocarbons. Metals present were below contamination levels.

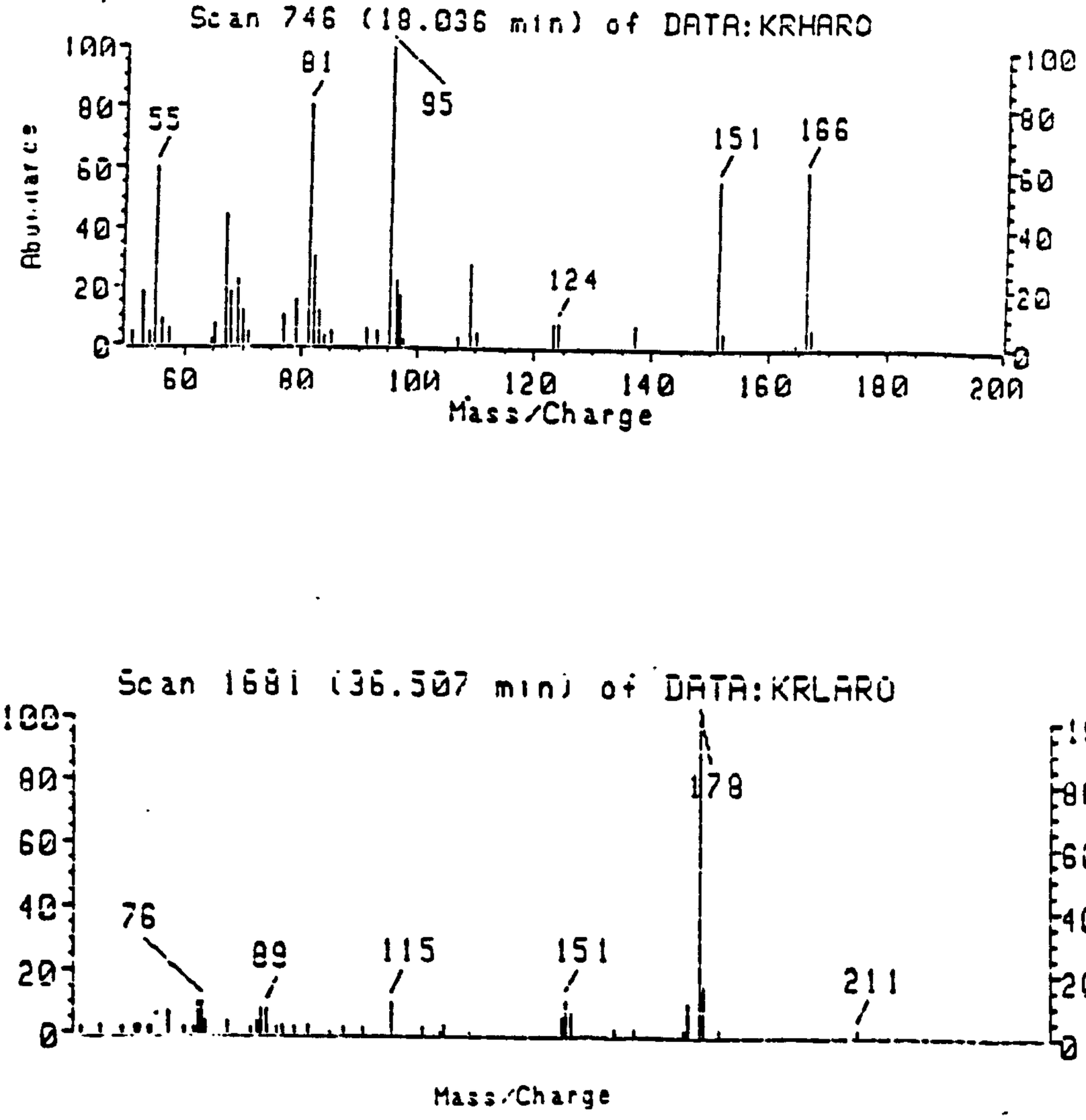


Figure 31. Mass spectra assigned to dimethylnaphthalene (top) and phenanthrene (above).

Mass spectra of dimethylnaphthalene (top) and phenanthrene (above) assigned on the basis of comparison with reference spectra held in computer data banks.

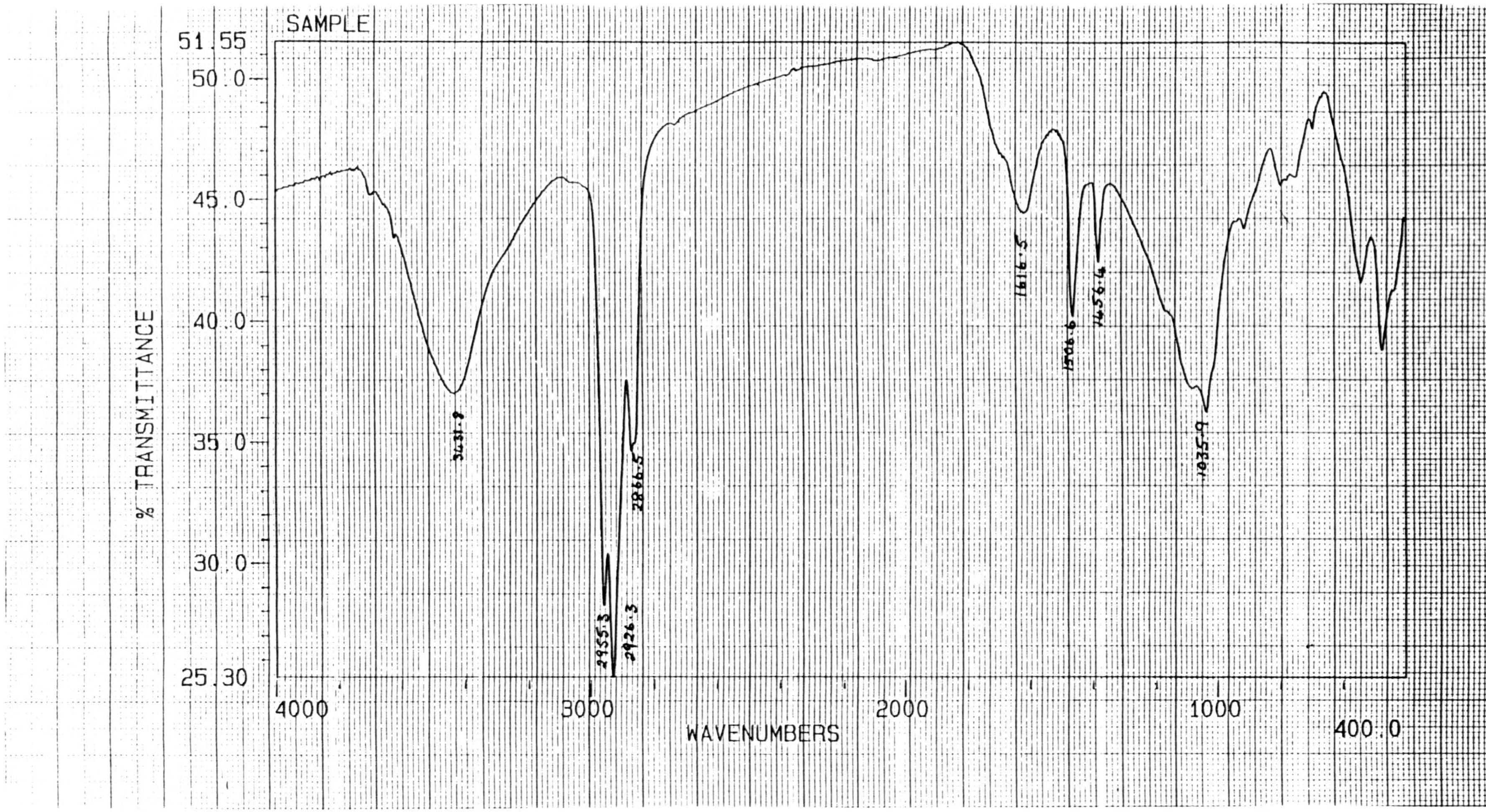


Figure 32. Infra red scan of Derwenthaugh soil in KBr disc.

A sample of sieved (>2mm) oven-dried Derwenthaugh soil was run as potassium bromide-admixed disc (ratio of sample to KBr = 1:300), on a Nicolet 20 PCIR.

4.3 Plant growth

Barley was grown on DWH soil as a bioassay and indication of the toxicity of the soil. The DWH soil was mixed with soil compost giving a concentration of 0-100% in pots (15cm diam.)

Emergence rates fell as the amount of DWH soil in pots containing both soil compost and the DWH soil was increased (Table 19). Nevertheless there was as much as 67% emergence of barley in pots containing 75% DWH soil. Mean growth rates and yield were severely depressed in all pots containing DWH soil; even though the majority of seeds germinated, the seedlings died within a few days of emergence. Mean growth rates and yield were significantly different ($p < 0.05$) between barley grown on soil compost alone and barley grown on 75% soil compost and 25% DWH soil. Plants were harvested at 3 weeks as barley grown even on 75% soil compost and 25% DWH soil was dying (Fig. 33).

4.4 Microbiological analyses

Soil contaminated with organic pollutants has been successfully remediated with the use of microbes (see Introduction p29). The Zurich test was undertaken to give an indication of the potential biodegradability of the lipophilic material in the DWH soil.

4.4.1 Zurich test

Hydrocarbon loss was established by comparing the oil content of the replicate experimental flasks. These flasks were exposed to a sewage inoculum or an inoculum enriched from Derwenthaugh soil, with the initial oil content of identical replicate flasks at zero time, after subtracting the abiotic loss value.

Over the three weeks the test was performed, only a small percentage of the i.r.-recorded hydrocarbon material extractable from the DWH soil was degraded by

Table 19. Mean emergence rates of spring barley grown on mixtures of Derwenthaugh soil and commercial soil compost.

Spring barley (*Hordeum vulgare*) was grown under glass in pots (15cm diam.) in mixtures of Derwenthaugh soil and soil compost in the following percentage of Derwenthaugh soil: 100; 75; 66; 50; 33; 25; 0. The percentage emergence of the plants was determined for each pot of three plants and the mean value calculated.

Derwenthaugh soil in mixture (%)	Emergence rate* of barley (%)
100	11
75	67
66	78
50	89
33	100
25	100
0	100

*Mean of 9 seeds in each mixture measured after 7 days at 20°C and 15% water holding capacity.

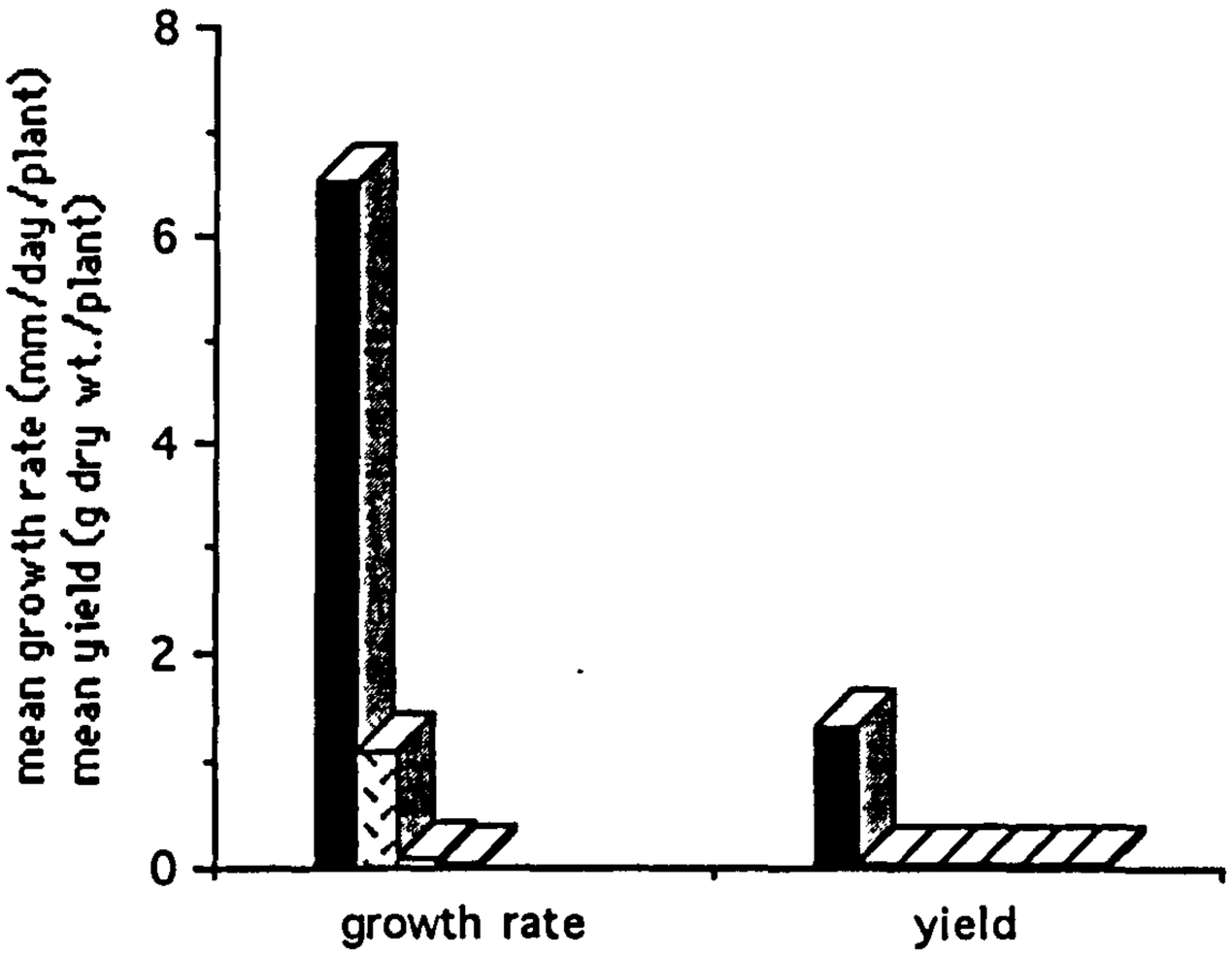


Figure 33. Growth rate and yield of barley grown on mixtures of Derwenthaugh soil and commercial soil compost.

Spring barley (*Hordeum vulgare*) was grown under glass in pots (15 diam.) in mixtures of Derwenthaugh soil and soil compost in the following percentage of Derwenthaugh soil (from L to R): 0 ■; 25 ▨; 33 ▨; 50 ▢; 66 ▥; 75 ▨; 100 ▤. The mean growth rate was determined by measuring the extension of the first leaf of each plant 10 and 17 days after seed germination. Plants were harvested at 3 weeks and mean dry weight exc. roots determined. Standard deviations were all below ± 0.5.

sewage inoculum; 1.5% by Day 7; 2.7% by Day 14; and 6.1% by Day 21. A slightly larger percentage of the hydrocarbon material was degraded by the DWH soil inoculum, pregrown on cresol; 5.8% by Day 7; 11.4% by Day 14; and 16.2% by Day 21. Abiotic loss from both sets of poisoned flasks was minimal. The rape seed oil used as a positive control and as calibration material, was completely degraded in 7 days (Table 20).

4.4.2 Microbial content of Derwenthaugh soil

Microbial counts of the Derwenthaugh soil were made to determine the viability of bacteria within the soil. Conditions for biodegradation were optimised and minerals added to try and stimulate the biodegradation of phenols. Bacteria enriched from Derwenthaugh soil were grown in a chemostat on phenols and a range of methylated phenols and thiocyanate, and subsequently added back to the soil.

Soil was stored at 4°C for 20h before microbial counts were made. Bacteria/g DWH soil were extracted and grown on plates of nutrient agar, nutrient agar diluted with mineral salts agar (1:5) and mineral salts agar containing *para*-cresol (2mM) as a carbon source. The mean number of bacteria/g fresh weight soil revealed on these media were as follows (Table 21):

nutrient agar	$2.70 \pm 2.8 \times 10^{6*}$
diluted nutrient agar	$1.04 \pm 0.7 \times 10^{7*}$
mineral salts agar + *p*-cresol	$1.05 \pm 1.0 \times 10^{6*}$

*values are means of nine replicates

The number of different organisms on each media averaged 7 for rich and diluted nutrient agar, and 5 on mineral salts agar + *p*-cresol. Both the total viable count and the diversity of organisms were highest on the diluted nutrient agar.

In conclusion, the hydrocarbon content of the DWH soil appeared resistant to biodegradation by sewage and DWH soil inoculum over 21 days with only 6.1% and 16.2% respectively of total hydrocarbon content being mineralised. Numbers of

Table 20. Residual oil content and biodegradability of a hydrocarbon extract from Derwenthaugh soil by a mixed raw sewage inoculum and an inoculum enriched from Derwenthaugh soil.

Flasks (250ml) contained 75ml of mineral salts medium with 1ml (10^6 cells) of inoculum (neutral flasks); with 25μl of an extract of Derwenthaugh (DWH) soil (test flasks); with 25μl of rape seed oil in CCL_4 (calibration flasks). Poisoned flasks (containing test or calibration material) were prepared as for either test or calibration flasks without the inoculum but with mercuric chloride solution (1ml of a 1g/100ml solution). The inoculated cultures were incubated with shaking at 180rev/min at 25°C in the dark and sampled for residual hydrocarbons by i.r. analysis at 0, 7, 14 and 21 days.

Days	Calibration residual oil content %	Biodegradability %	Test$^{=*}$ residual oil content % sewage inoculum	Biodegradability %^{v}	Test$^{=*}$ residual oil content % DWH inoculum	Biodegradability %^{v}
0		100	100	0.0	100	0.0
7	0	100	97.5±1.5	1.5	93.2±1.0	5.8
14	0	100	96.3±1.5	2.7	87.7±1.5	11.4
21	0	100	93.0±2.6	6.1	83.0±1.7	16.2

$^{=}$ Poisoned flasks (both calibration and test had residual oil content of 100% and 99% respectively)

* Results are mean of three replicates

^{v} Biodegradability (%) = $\frac{P - T}{P} \times 100$

where P = Residual oil content of poisoned flask in % (mean values)

and T = Residual oil content of test flasks in % (mean values)

Table 21. Viable counts on Derwenthaugh soil.

Soil (5g fresh weight) was mechanically blended with sodium cholate solution. Iminodiacetic acid chelating resin and 30 glass beads were added to the soil samples which were shaken end-over-end for 2h at 5°C and centrifuged at 2033 rev/min for 30sec. The soil pellets were resuspended in various solutions, shaken and centrifuged as detailed in Section 2.6.1. All supernatants were pooled, diluted and plated onto nutrient agar, diluted nutrient agar (1:5) and mineral salts agar + *p*-cresol. The number and type of organisms per plate (three replicates of each dilution) were counted and the mean results are indicated below.

Dilution factor	Number of organisms (mean)	Number of types (mean and range)	Number of organisms/ml original suspension	Number of organisms/g soil
Nutrient agar				
10^4	29.8±8.7	5.5 (4-7)	14.9×10^5	5.9×10^6
10^3	92.7±26.4	7.4 (7-8)	4.6×10^5	1.8×10^6
				mean: $2.7 \pm 2.8 \times 10^6$
Diluted nutrient agar (1:5)				
10^5	9.1±11.6	3.2 (1-5)	4.5×10^6	18.0×10^6
10^4	46.8±14.9	6.0 (4-8)	2.3×10^6	9.2×10^6
10^3	203.0±11.8	7.1 (5-11)	1.0×10^6	4.0×10^6
				mean: $1.04 \pm 0.7 \times 10^7$
Mineral salts agar + *p*-cresol				
10^4	22.0±4.3	4.0 (4)	11.0×10^5	4.4×10^5
10^3	116.0±58.6	5.4 (4-7)	5.8×10^5	23.0×10^5
				mean: $1.05 \pm 1.0 \times 10^6$

bacteria present in that soil were low but $1x10^6$ organisms/g soil were capable of degrading *para*-cresol.

4.4.3 Enrichment of trimethylphenol-degrading and cresol-degrading cultures from Dunston soil, River Tyne dredgings and Derwenthaugh soil.

Cultures capable of degrading trimethylphenols were enriched from the Dunston coke work soil. Both 2,4,6-and 3,4,5-trimethylphenol substrates (0.5mM) were degraded completely within 2 weeks (Fig. 34 and 35) in such enrichments. Similar enrichment cultures using 2,3,5-trimethylphenol showed only a 45% loss of the 2,3,5-isomer in 3 weeks (not illustrated). Linear regression analyses of plots of $\log_{10}$ (% trimethylphenol remaining) *vs* incubation time showed that rate constants (k values) were concentration dependent, with a decrease in k values as the concentration of trimethylphenol was increased from 0.5-2.0mM in the cultures. There was a 2-fold difference in the decay constant for 2,4,6-trimethylphenol disappearance between flasks containing 0.5mM ($k=-0.14\ days^{-1}$) and 2.0mM ($k=-0.06\ days^{-1}$) [Appendix 1 (i)] and an 14-fold difference in the constant for 3,4,5-trimethylphenol disappearance between flasks containing 0.5mM ($k=-0.14\ days^{-1}$) and 2.0mM ($k=-0.01\ days^{-1}$) [Appendix 1 (ii)].

Enrichment cultures, selected for their capacity to degrade *para*-cresol were originally set up using the River Tyne dredgings as preliminary reports indicated phenols present in the dredgings were above the threshold levels. When it became apparent that the original phenols present were significantly depleted by `weathering` the dredgings to air, attention was switched to the Derwenthaugh soil which was heavily polluted with phenolic compounds.

Cresol-degrading enrichment cultures were obtained readily from River Tyne dredgings and Derwenthaugh soil. Bacteria in Tyne river water and River Tyne dredgings degraded cresol (mixed isomers, 500μM) within 5 days when inoculated into

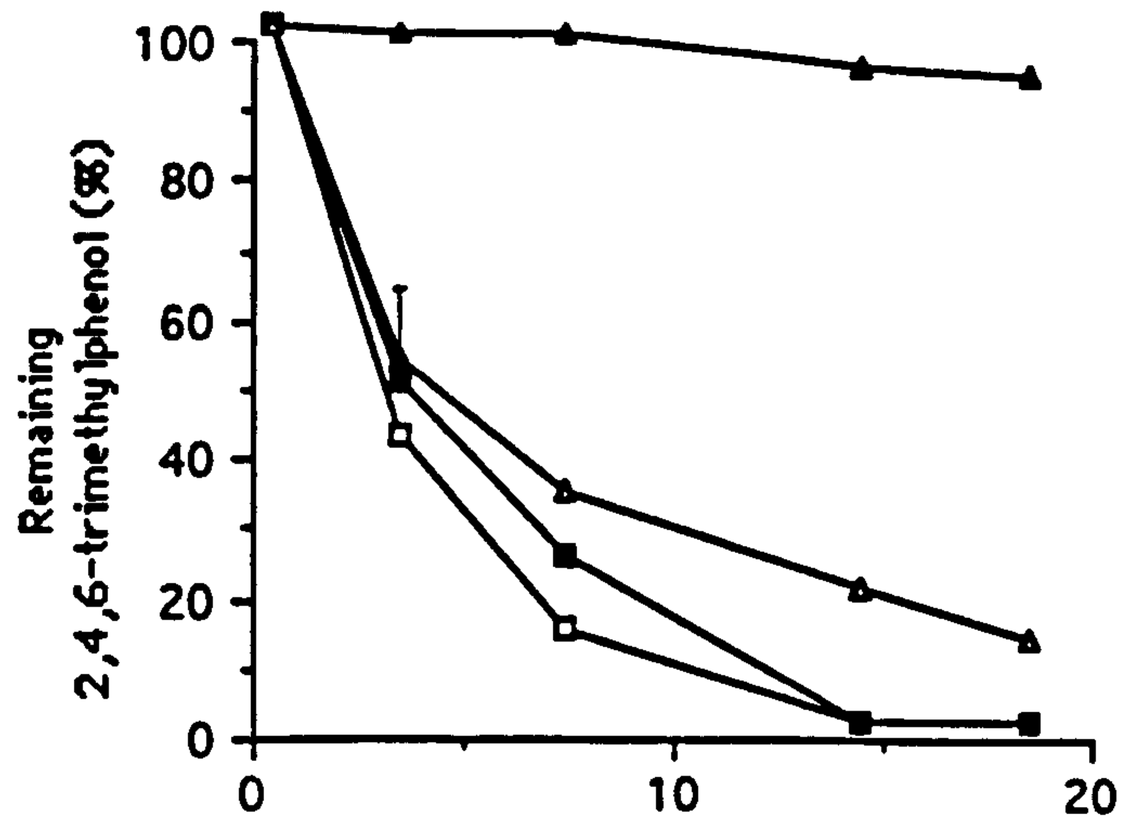


Figure 34. Degradation of 2,4,6-trimethylphenol by an enrichment culture.

Flasks (100ml) containing 50ml mineral media were supplemented with 2,4,6-trimethylphenol at 0.5mM (❑); 1.0mM (■) or 2mM (Δ) and inoculated with 1ml of an exponentially-growing culture on 2,4,6-trimethylphenol, raised from the derelict coke work site at Dunston. Uninoculated flasks (▲) served as controls. Flasks were incubated at 25°C, in the dark, shaking at 200rev/min. Residual 2,4,6-trimethylphenol was determined by HPLC. Standard deviations are shown by error bars where variation between replicates (3) was above 5%.

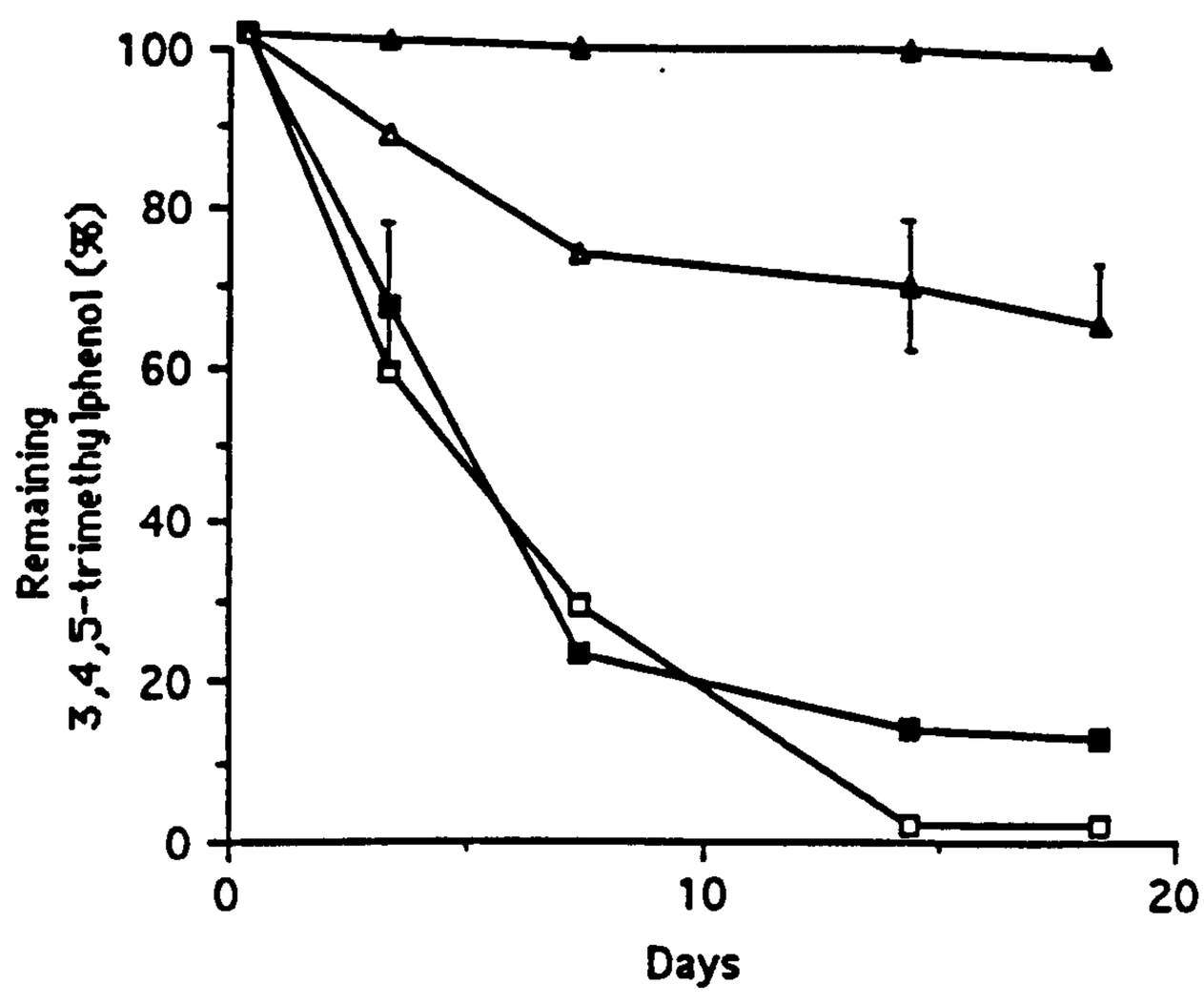


Figure 35. Degradation of 3,4,5-trimethylphenol by an enrichment culture.

Flasks (100ml) containing 50ml mineral media were supplemented with 3,4,5-trimethylphenol at 0.5mM (□); 1.0mM (■) or 2mM (Δ) and inoculated with 1ml of an exponentially-growing culture on 3,4,5-trimethylphenol, raised from the derelict coke work site at Dunston. Uninoculated flasks (▲) served as controls. Flasks were incubated at 25°C, in the dark, shaking at 200rev/min. Residual 3,4,5-trimethylphenol was determined by HPLC. Standard deviations are shown by error bars where variation between replicates (3) was above 5%.

cultures from either 1g sediment or 10ml Tyne water (Fig. 36). Enrichment cultures raised from the Derwenthaugh coke works also had the ability to degrade 0.5mM cresol totally within 72h without the accumulation of u.v.-absorbing metabolites; at higher concentrations of cresol (2mM) the culture supernatant became pale yellow in colour. The yellow colour observed in the chemostat culture (Photograph 14) was thought to be indicative of the hydroxylation of cresol to methylcatechol and possible fission of the latter by meta cleavage.

The culture showing the greatest potential for cresol degradation with concomitant increase in cell numbers in batch enrichment was the Derwenthaugh enrichment which was subsequently used as the inoculum for continuous culture experiments set up using a chemostat. After steady state was achieved (approximately 3 volume changes), the level of cresol was monitored every week and gradually increased in 1mM steps (every 10 days) until the culture began to wash out at 6.5mM. Thus the level of cresol was reduced to 6.0mM and the culture kept in steady state at that level. Slight frothing of the culture was noted at higher concentrations of cresol.

The chemostat was fed with 6mM cresol (mixed isomers) and sampled daily over a period of weeks; when HPLC results indicated that the residual cresol concentration in the fermentation vessel was consistently negligible, potassium thiocyanate as KSCN at 0.5mM was added to the growth media. The concentration of thiocyanate remained constant while ammonium was present in the mineral salts feed, but when the latter was removed, thiocyanate acted as the sole source of nitrogen and was depleted. When both ammonium and thiocyanate were removed from the growth media for 92h, cresol degradation decreased dramatically. The amounts of thiocyanate and cresol utilised were inter-dependent. When varying ratios of cresol:thiocyanate were used in the growth medium, a ratio of 3mM cresol:1mM thiocyanate was required before all the thiocyanate in the chemostat was removed (Fig. 37).

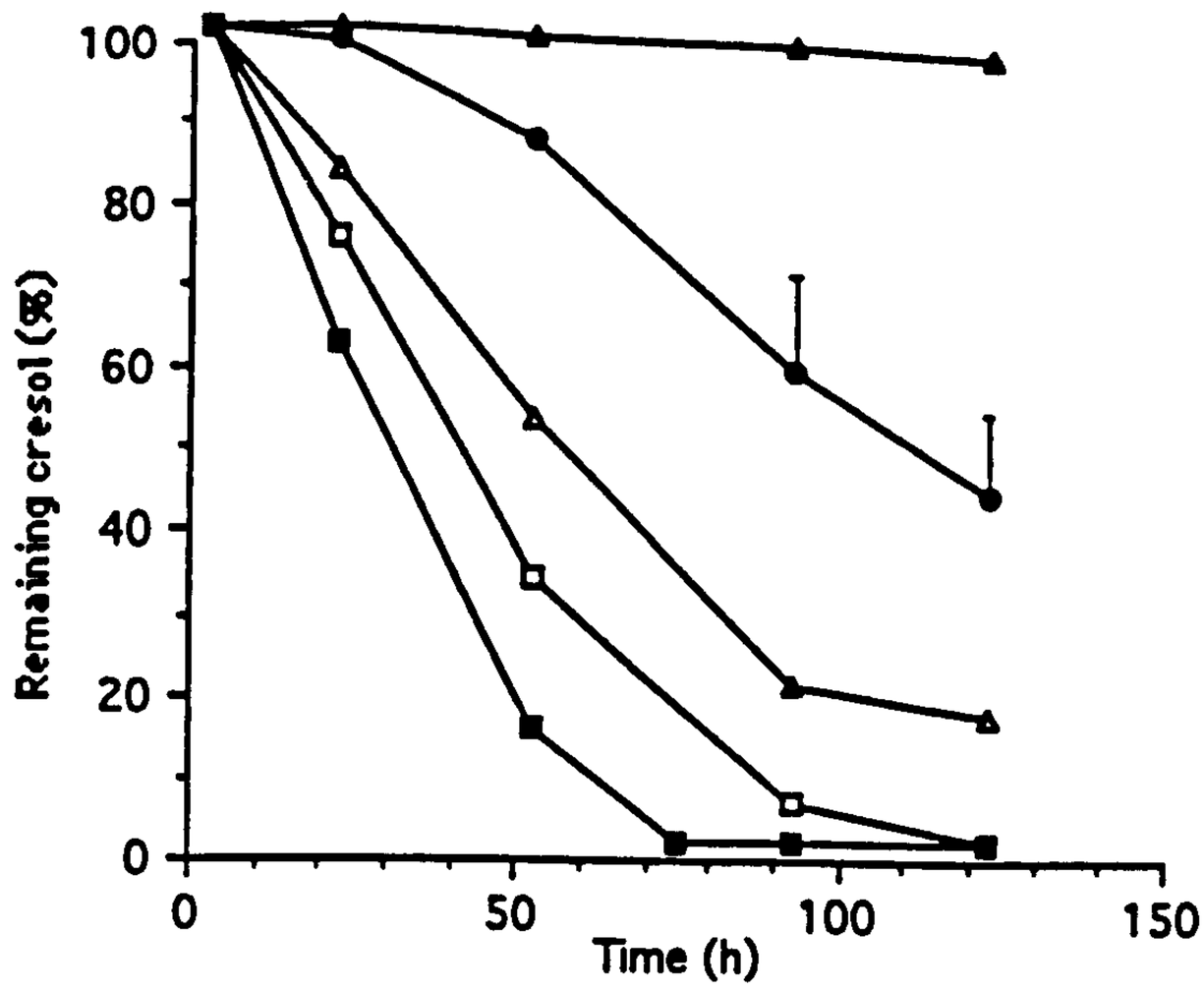
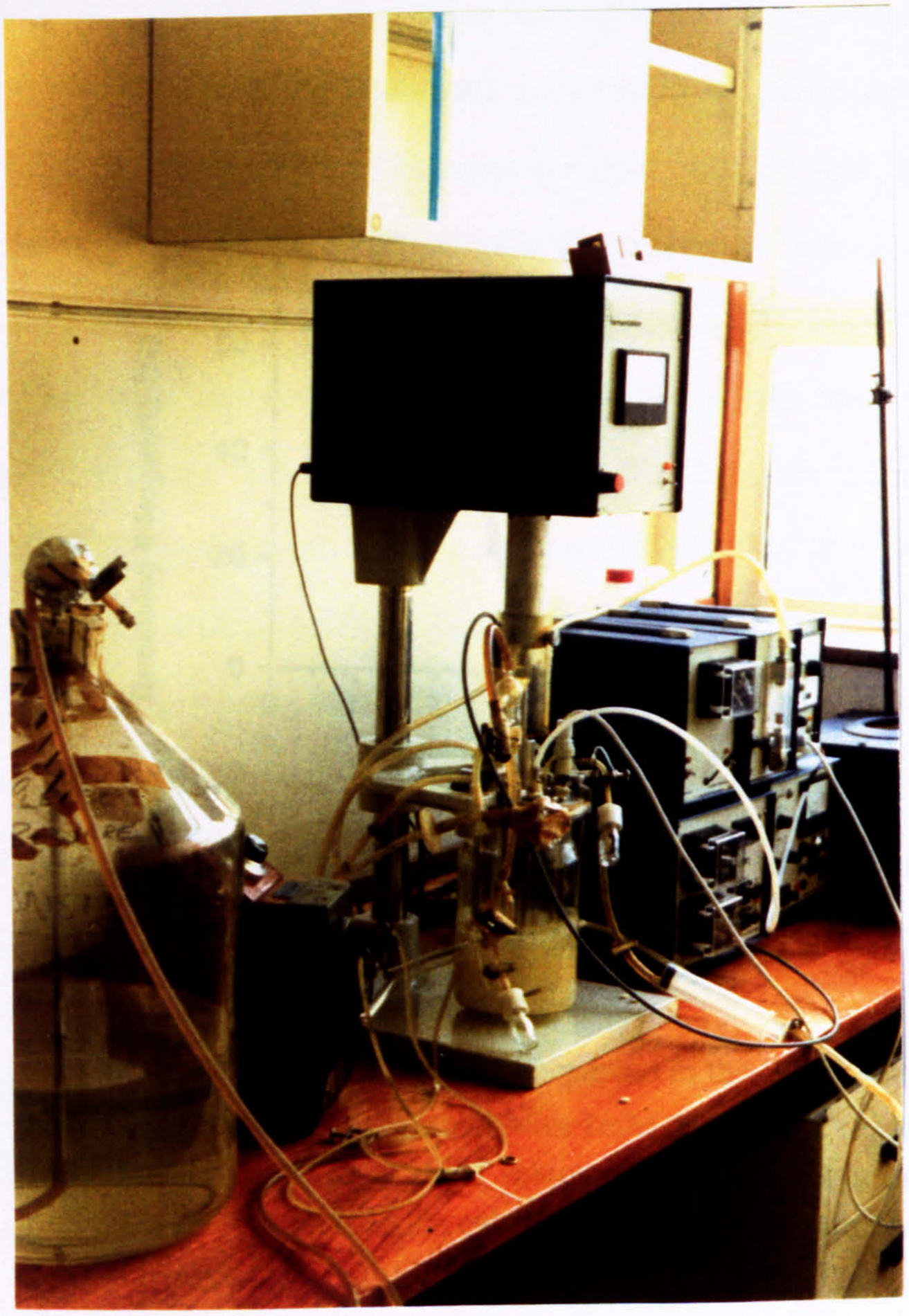


Figure 36. Degradation of cresol by enrichment cultures.

Flasks (100ml) containing 50ml mineral media with cresol (0.5mM) were inoculated with either: 1g of River Tyne sediment dredged in 1986 (●); 1g of River Tyne sediment dredged in 1987 (Δ); 10ml River Tyne water (❑); 1g of Derwenthaugh soil (■) or left uninoculated (▲) as a control. Flasks were incubated at 25°C, in the dark, shaking at 200rev/min. Cresol was determined by HPLC. Standard deviations are shown by error bars where variation between replicates (3) was above 5%.



Photograph 14. Continuous enrichment cultures growing on cresol (5mM) + thiocyanate (2mM).

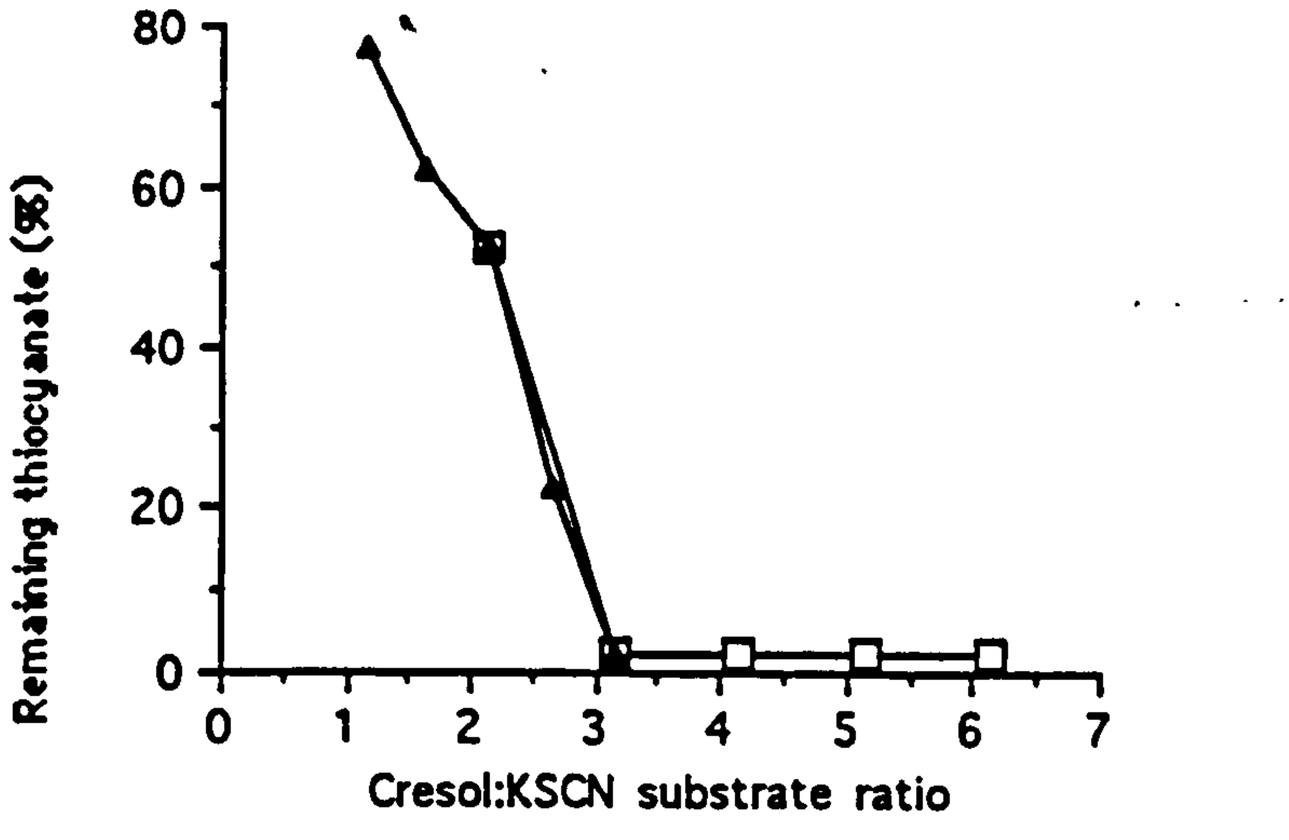


Figure 37. Percentage of residual thiocyanate in chemostat grown on different substrate levels.

The substrate ratio of cresol:thiocyanate in the chemostat was varied from: a) 2mM cresol to 6mM cresol with 1mM thiocyanate (▲) and b) 2mM cresol to 6mM cresol with 2mM thiocyanate (❑), over a period of months. Thiocyanate present in the culture supernatant was estimated by colorimetric analysis (A.P.H.A., 1975).

4.4.4 Growth of the chemostat enrichment in batch cultures on cresol (mixed isomers) and thiocyanate

The mixed culture from the chemostat grew in batch cultures at the expense of cresols and thiocyanate with cell densities increasing as cresol removal occurred. With an inoculum grown on cresol and thiocyanate there was an immediate rapid degradation of cresol. When the inoculum was grown previously on nutrient agar, there was a lag of one or two days before the onset of rapid degradation of cresol. At 25°C with shaking (150-200rev/min) the time for total degradation of 2.5mM cresols was 64h at which time maximum cell numbers were also obtained. Under these conditions the specific growth rate (μ) was $0.1h^{-1}$ (t_d approx. 21h). KSCN levels fell from 1.5mM to approximately 0.4mM within the same time. If the initial KSCN level was reduced to 1.0mM. it was completely utilised together with the cresols in 60-80h, with a concurrent increase in sulphite and sulphate concentrations in the growth media (Fig. 38). When ammonium chloride (0.2mM) was added to the flasks at 40h, KSCN utilisation ceased and sulphite and sulphate levels remained constant within the growth media (Fig. 38).

4.4.5 Identification

Microscopic examination of the enriched mixed culture from the chemostat revealed it was purely bacterial in nature. Colonies were quite distinctive when grown and took 48h to develop on nutrient agar. Two of the larger colony forms, B and D, were both regularly shaped, convex, and white or opaque respectively in colour. The organisms giving these colonies remained constant in the chemostat under different growth conditions. The other two isolates were detected periodically. All isolates were catalase-positive and oxidase-positive. The API 20NE rapid identification kit for non-enteric Gram-negative rods was used for the majority of biochemical tests carried out on the isolates. Biochemical differentiation suggested that three of the organisms in the

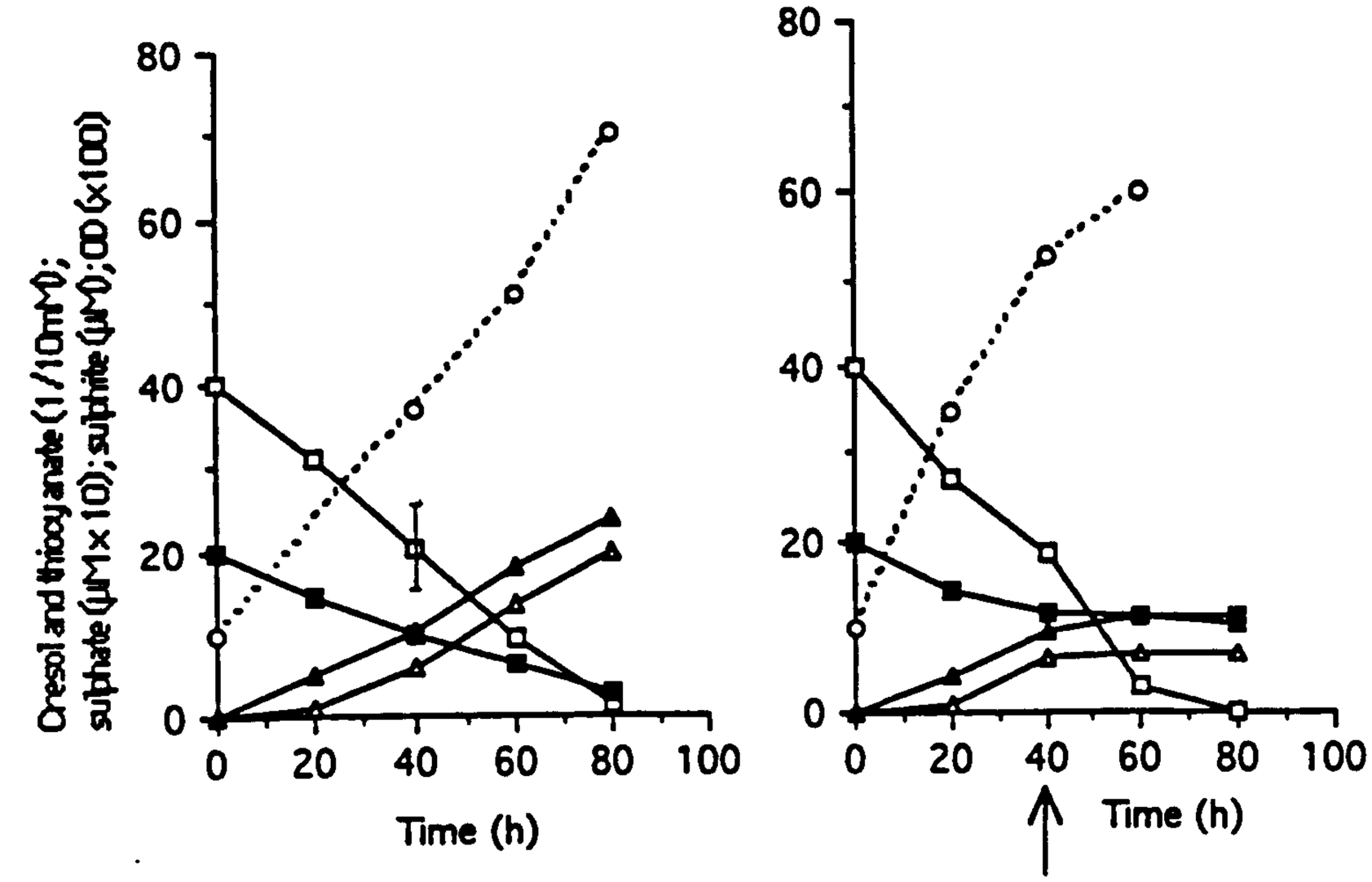


Figure 38. Degradation of cresol and thiocyanate.

Flasks (100ml) containing 50ml mineral media with cresol (3.0mM) and thiocyanate (1.0mM) were inoculated with 1ml of an exponentially-growing culture on cresol and thiocyanate from a chemostat enrichment. The inoculated cultures were incubated at 25°C, in the dark, shaking at 200rev/min and sampled for: growth at A_{540} (o); residual cresol (❑); thiocyanate (■); sulphite (Δ); and sulphate (▲), at regular intervals. Nitrogen in the form of ammonium chloride was added as indicated by arrow (rt. hand graph). Standard deviations are shown by error bars where variation between replicates (3) was above 5%.

mixed culture belonged to the genus *Pseudomonas*, with organisms `B` and `D` identified as *Pseudomonas stutzeri* and *Pseudomonas fluorescens* respectively (Table 22).

Species identification was confirmed by fatty acid composition analysis of the chemostat isolates. Cellular fatty acids isolated from the cresol-degrading bacteria were analysed as their fatty acid methyl esters (FAMEs). The major fatty acids were identified from equivalent chain length (ECL) calculations (Gillan, 1983), using the retention time of FAMEs of known even- and odd-numbered straight chain saturated fatty acids. Figs. 39 and 40 illustrate the g.c. traces of the FAMEs of standard fatty acids and those obtained from cells of the mixed culture. The fatty acid patterns of cultures D and B in which even-numbered straight chain saturated, monosaturated acids and hydroxylated fatty acids were present confirmed that they belonged to the genus *Pseudomonas* (Jantzen & Bryn, 1985). Odd-numbered chain fatty acids were also present but only in small amounts (Table 23).

4.5.6 Utilisation of catechols by the chemostat-enrichment cultures

When a cresol-grown inoculum from the chemostat was used, catechol was completely removed without a lag in 40h in batch cultures. 4-Methylcatechol was subsequently removed in 46h and 3-methylcatechol in 85h. There was a 10% loss (approx) of catechols in both dead cell and abiotic controls after 80h. (Fig. 41). When all three catechols were present together in a mixed culture, the same pattern of degradation was evident.

Table 22. Biochemical characteristics of organisms that comprised the chemostat mixed culture.

	A	B	C	D
Colonial form	convex yellow	convex white	irregular cream	convex opaque
Gram stain	Gm- rods	Gm- rods	Gm- rods	Gm- rods
Catalase	+	+	+	+
Oxidase	+	+	+	+
NO_3 reduction	-	-	-	+
Indole	-	-	-	-
Acid from glucose	-	-	-	-
Arginine	-	-	+	+
Urease	-	-	-	-
Aesculin hydrolysis	+	-	-	-
Gelatin hydrolysis	+	+	-	-
B-galactosidase	-	-	-	-
Carbon source assimilation				
Glucose	+	+	+	+
Arabinose	+	+	-	+
Mannose	+	+	-	+
Mannitol	+	+	+	+
N-Acetylglucosamine	+	-	+	+
Maltose	+	+	-	-
Gluconate	-	+	+	+
Caprate	-	-	-	+
Adipate	-	+	+	-
Malate	-	+	+	+
Citrate	+	+	+	+
Phenylacetate	+	-	+	+

Identification

Culture A Flavobacterium
Culture B *Pseudomonas stutzeri*
Culture C *Pseudomonas* sp.
Culture D *Pseudomonas fluorescens*

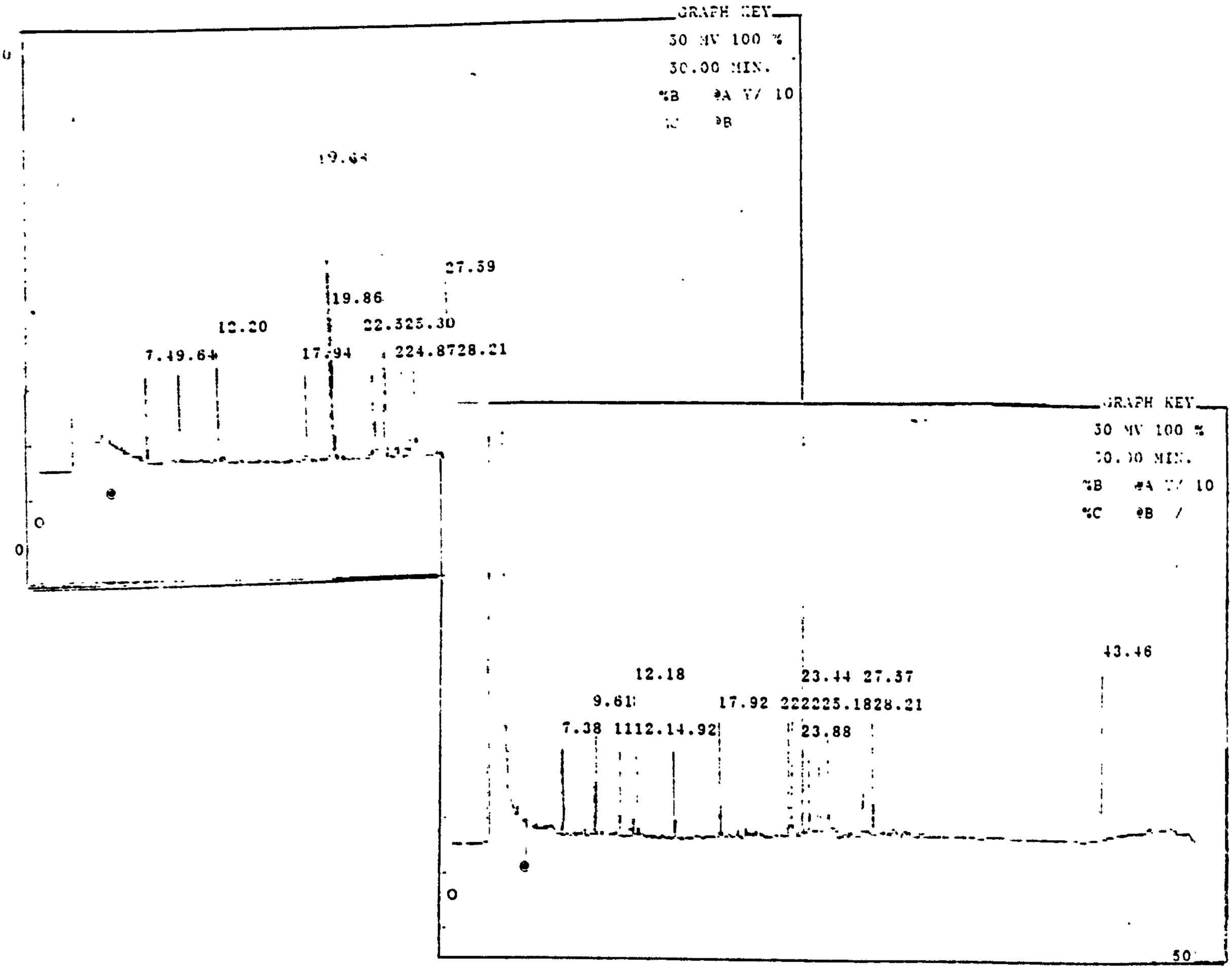


Figure 39. Fatty acids from two organisms from mixed chemostat culture.

Retention times of fatty acid methyl esters (FAME`s) extracted and prepared from chemostat cultures B (top), D (above) and of FAME standards (overleaf) were obtained experimentally. Fatty acid moieties were identified from the ECL values quoted by Gillan (1983).

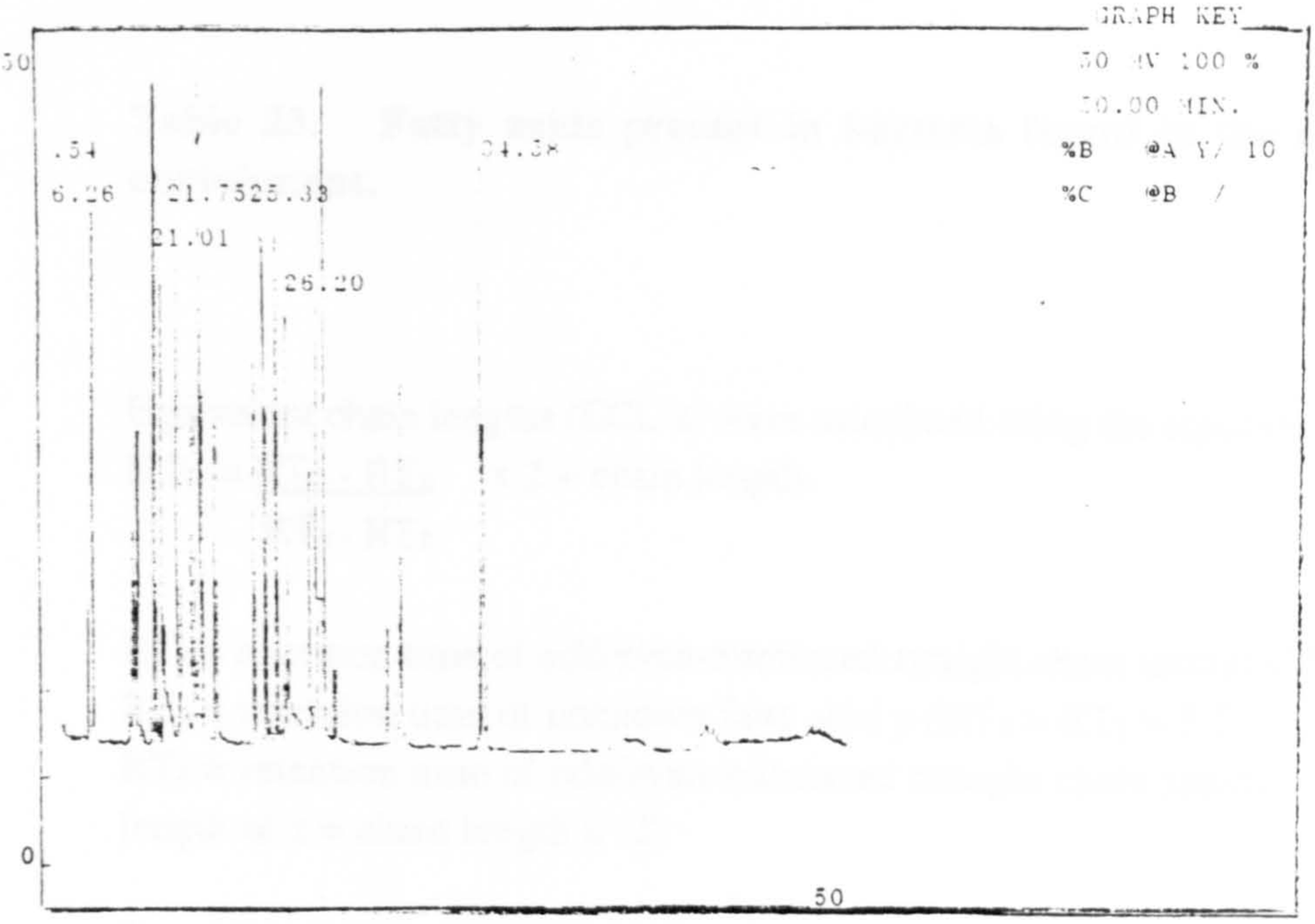


Figure 40. Fatty acids present in standard mix.
Gas chromatogram of fatty acid methyl esters (FAMEs) of standard straight chain fatty acids (C_{10}-C_{20}).

retention time	fatty acid	retention time	fatty acid
7.23	11.0	21.75	i16:0
10.12	2OH-10:0	22.40	16.1 9
10.28	12:0	22.70	16:0
12.64	13:0	23.31	i17:0
15.31	2OH-12:0	24.94	17:0D
15.54	3OH-12:0	25.33	17:0
16.26	14:0	25.85	2OH-16:0
18.03	i15:0	27.28	a18:0
19.75	a15:0	27.77	19:0D
19.96	15:0	31.09	19:0
20.72	2OH-14:0	34.58	20:0
21.01	3OH-14:0		

Fatty acids are denoted as N:M x where N = number of carbon atoms, M = number of double bonds and x = position of nearest double bond to carboxyl. OH = hydroxylated fatty acid, 2 and 3 indicate position of hydroxyl group, i = iso-branched fatty acid, di = dicarboxylic acid, a = anteiso-branched fatty acid, and D = D isomer.

Table 23. Fatty acids present in bacteria found in the chemostat enrichment.

Equivalent chain lengths (ECL`s) were calculated using the equation:

$$ECL = \frac{RT_x - RT_z}{RT_y - RT_z} \times 2 + \text{chain length}_z$$

RT_x = retention time of odd/even-numbered straight chain saturated fatty acid x.
RT_y = retention time of unknown fatty acid y ($RT_x > RT_y > RT_z$).
RT_z = retention time of odd/even-numbered straight chain saturated fatty acid z (chain length of z = chain length x -2).

Retention times of fatty acid methyl esters (FAME`s) extracted and prepared from chemostat cultures and of FAME standards were obtained experimentally. Fatty acid moieties were identified from the ECL values quoted by Gillan (1983).

Retention time (min)	ECL_{calc}	ECL_{lit}	$FAME_{lit}$	%
Culture B				
19.70 ±0.024	15.02	15.02	15:0	16.0
23.35 ±0.037	17.95	18.0	18:0	53.0
27.59 ±0.028	19.1	19.14	di 16:0	9.0
Culture D				
23.36 ±0.031	17.93	18.0	18:0	60.0
24.58 ±0.026	17.16	17.0	17:0	5.0
27.57 ±0.034	19.15	19.14	di 16:0	7.0

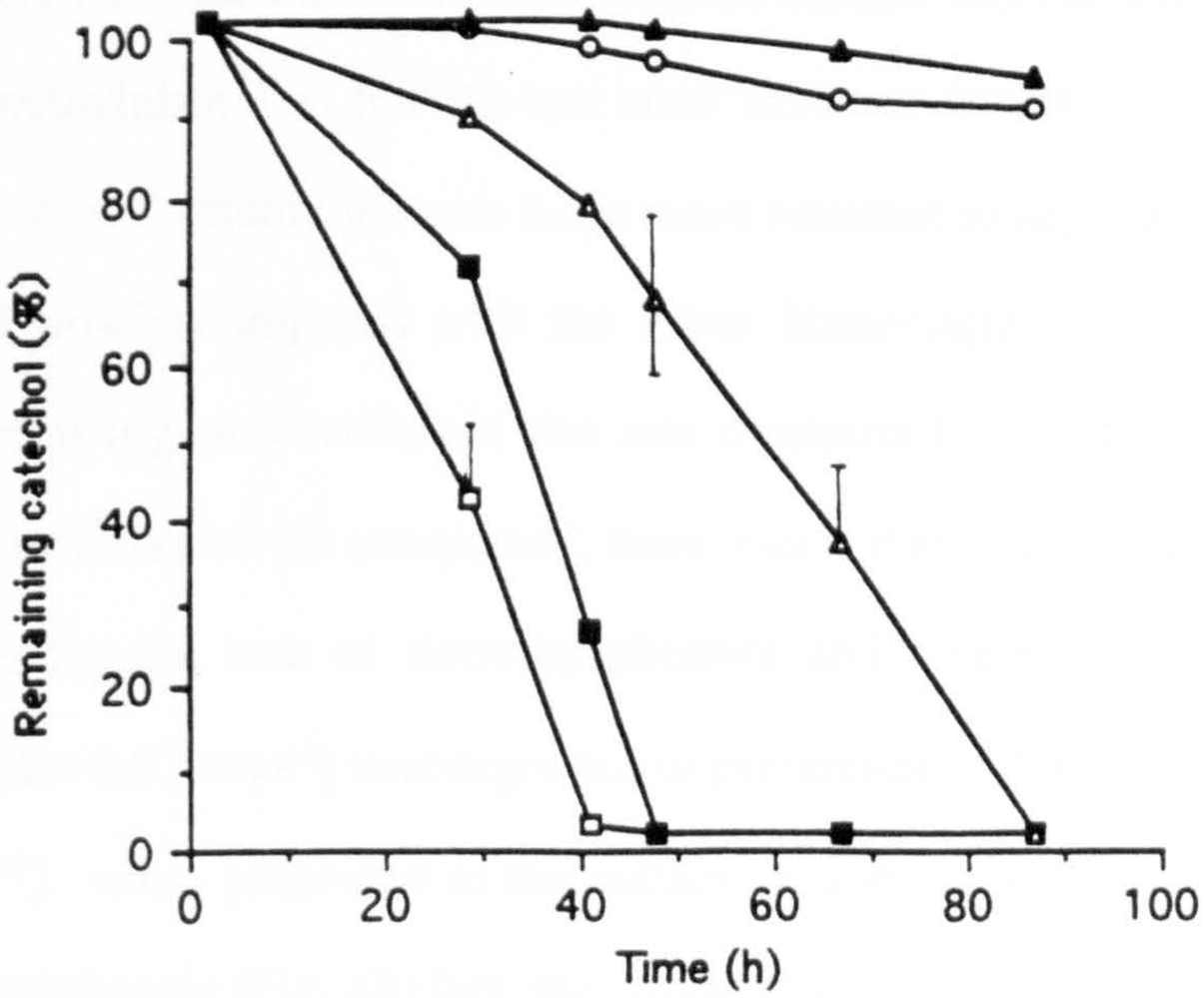


Figure 41. Degradation of catechols by a mixed culture.

Flasks (100ml) containing 50ml mineral media with thiocyanate (1.0mM) were supplemented with either catechol (2mM) (❑); 3-methylcatechol (2mM) (Δ); or 4-methylcatechol (2mM) (■); and inoculated with exponentially-growing chemostat cells, previously grown on cresol and thiocyanate. Both uninoculated (▲) and dead cell controls (o) were used to measure the abiotic oxidation of the catechols. Flasks were incubated at 25°C, in the dark, shaking at 200rev/min. Catechols were determined by HPLC. Standard deviations are shown by error bars where variation between replicates (3) was above 5%.

4.5.7 Utilisation of cresol and related methylphenols by resting cell suspensions of the chemostat mixed culture

Resting cell suspensions of the chemostat-enriched mixed culture were capable of metabolizing a wide range of phenolic compounds, both when presented singly and together (Fig. 42 and 43). Degradation of cresol and other related methylated phenols by the mixed culture illustrated that the isolated mixed culture was capable of degrading cresol and 2,4-dimethylphenol within 24h and other isomeric dimethylphenols between 1 and 7 days (Fig. 42). Trimethylphenols were more resistant to degradation and were only metabolised slowly compared with the lower homologues. Linear regression analysis of the semi-log plot indicated the rate constant for phenol removal was dependent on the structure of the compound; there was a 100-fold difference between the rate constants for the loss of dimethylphenols and trimethylphenols. 2,4,6-Trimethylphenol ($k=-0.01$ days^{-1}) was degraded in preference to 3,4,5-trimethylphenol ($k=-0.0001$ days^{-1}) when presented to the culture in a mixture together with other mono- and dimethylphenols (Fig. 43) [see also Appendix 1 (iii) and (iv)].

4.5.8 Oxidation of cresol and related methylphenols by whole cells of the chemostat mixed culture

Only a narrow range of compounds was oxidised significantly by the chemostat mixed culture, grown previously on cresol and thiocyanate. The cresols supported the best rates of oxygen consumption, followed by dimethylphenols. Methylphenols with substitutions in the 2- and 4-positions gave better oxidation rates than those compounds with substitutions in other positions (Table 24).

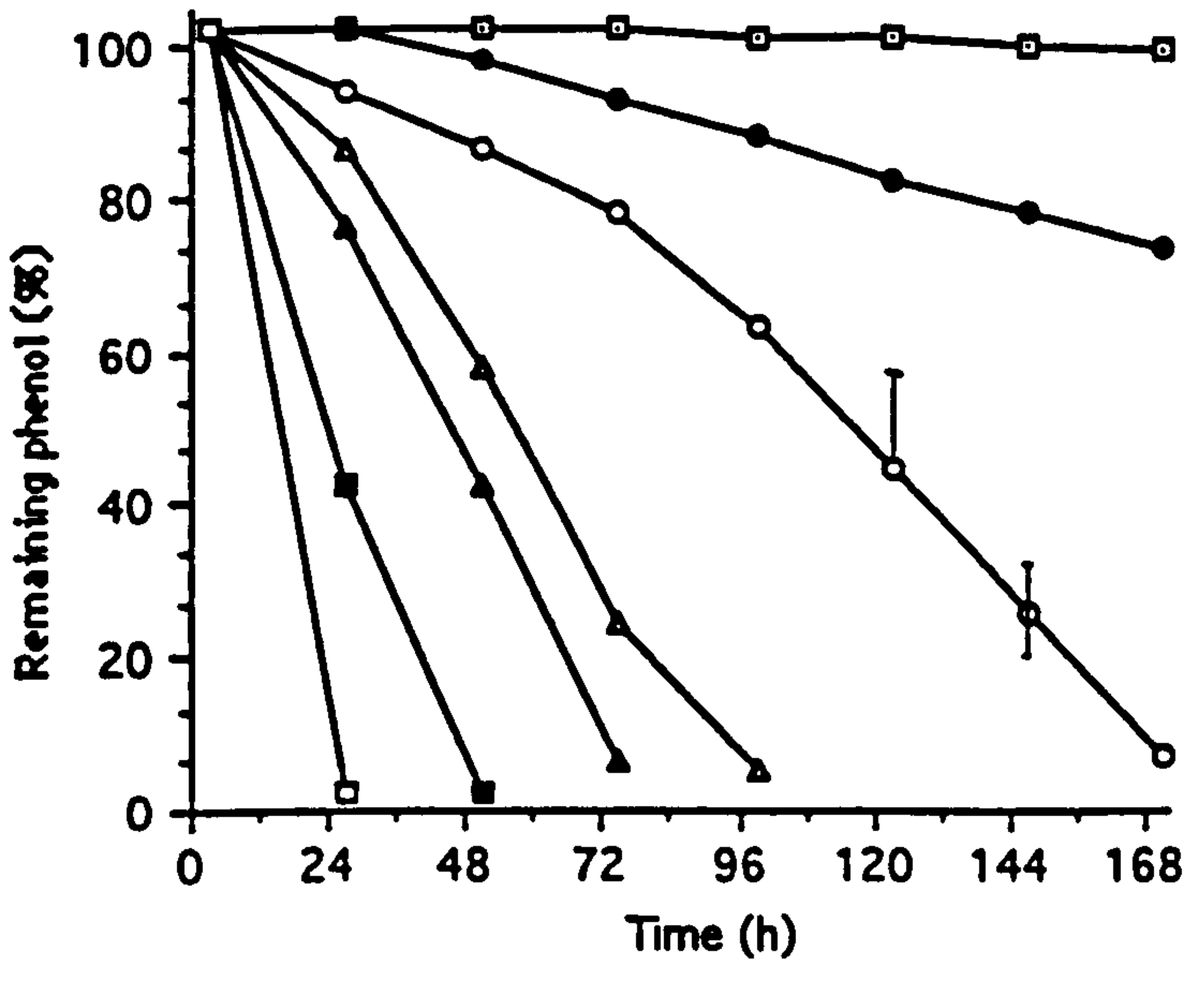


Figure 42. Degradation of a range of methylated phenols by a chemostat-enriched mixed culture.

Flasks (100ml) containing 50ml mineral media with thiocyanate (1.0mM) as a N-source were supplemented with either o,m,p-cresol; 2,4-dimethylphenol (❑); 3,4-dimethylphenol (■); 3,5-dimethylphenol (▲); 2,6-dimethylphenol; 2,3-dimethylphenol (Δ); 2,5-dimethylphenol (o); 2,4,6-trimethylphenol (●); or 3,4,5-trimethylphenol (⊡) added to each flask (2mM, final concentration) were inoculated with 1ml of washed exponential-phase cells previously grown on cresol and thiocyanate, suspended in phosphate buffer (25mM) to a density of 0.6mg/ml. Flasks were incubated at 25°C, in the dark, shaking at 200rev/min. Residual phenolics were determined by HPLC. Standard deviations are shown by error bars where variation between replicates (3) was above 5%.

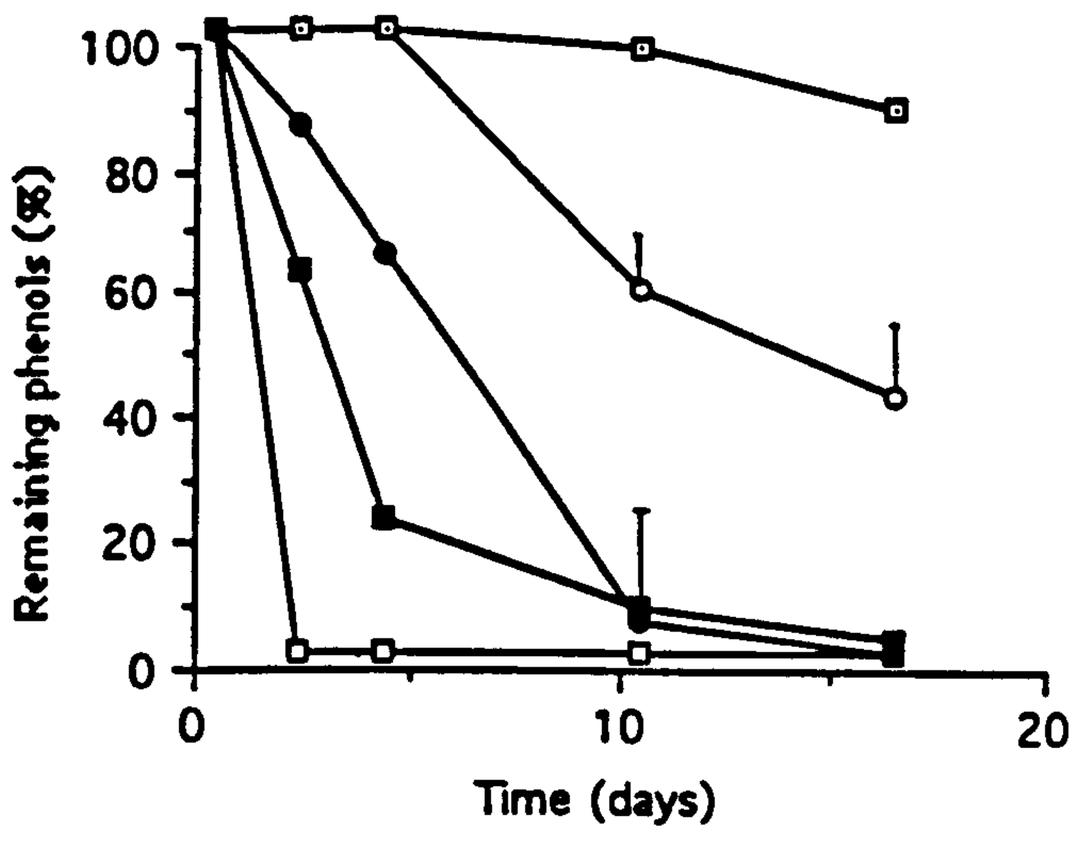


Figure 43. Utilisation of mixed phenols by the chemostat mixed-culture previously grown on cresol and thiocyanate.

Flasks (100ml) containing 50ml mineral media with thiocyanate (1.0mM) as a N-source and supplemented with p-cresol (❑); 3,4-dimethylphenol (■); 2,4,6-trimethylphenol (●); 2,5-dimethylphenol (o) and 3,4,5-trimethylphenol (⊡) all added to each flask (3mM, final concentration) were inoculated with exponentially-growing chemostat cells, previously grown on cresol and thiocyanate. Flasks were incubated at 25°C, in the dark, shaking at 200rev/min. Residual phenolics were determined by HPLC. Standard deviations are shown by error bars where variation between replicates (3) was above 5%.

Table 24. Oxidation of phenols by whole cells of the mixed culture after growth on cresol and thiocyanate.

Chemostat cultures were grown in the presence of cresol and thiocyanate, harvested, washed and resuspended in phosphate buffer (0.1M, pH 7). A sample of this suspension (equivalent to 0.4mg dry wt cells/ml) was added to 1ml buffer in the oxygen electrode cell, maintained at 25°C.
After temperature equilibration, the endogenous respiration rate of the suspension was measured [0.6μmol$_2$(min g dry wt)$^{-1}$] over a period of 3-5min and then 30μl of a 50mg/ml solution of the appropriate phenol was added to the suspension. The subsequent oxidation rates, shown below, are corrected for endogenous respiration.

Substrate	Rate (nmoles oxygen/min mg dry wt.) mixed culture	organism C	organism D
Phenol	69		26
o-Cresol	304		190
m-Cresol	140		45
p-Cresol	105		298
2,3-Dimethylphenol	43	6	22
2,4-Dimethylphenol	58	40	140
2,5-Dimethylphenol	27		6
2,6-Dimethylphenol	33		24
3,4-Dimethylphenol	77	25	62
3,5-Dimethylphenol	60		26
2,3,5-Trimethylphenol	12	2	17
2,4,6-Trimethylphenol	23	22	20

4.5.9 Biodegradation experiment

Analysis (Table 17) had shown the DWH soil to be heavily contaminated with coal tars, consisting of a complex mixture of aliphatic polycyclic and aromatic hydrocarbons and phenols. Metal contamination, in contrast to that of the River Tyne dredgings was not significant when measured as total and available metals (Table 18). From sampling at different depths of the DWH site it appeared that the contamination had occurred at the surface and had soaked into the soil (Table 25). Although it was not known exactly over how much time since contamination had occurred, a rough estimation would be about 35 years, as the coke works at Derwenthaugh were built in 1929 and closed in 1985.

Microbial viable counts of the Derwenthaugh soil had shown the presence of bacteria capable of degrading cresol and thiocyanate; indeed, enrichment cultures had been raised from it capable of degrading a wide range of phenols. An experiment was designed to test the effectiveness of adding additional laboratory-grown biomass (capable of degrading cresol and thiocyanate) to the soil and monitoring the subsequent disappearance of water-soluble phenolic compounds. Arable soil was added as an alternative inoculum in a parallel experiment; with the advantage that its addition would both dilute the existing contamination as well as provide mineral nutrients and improve soil aeration. It was extremely important that environmental factors favoured biodegradation. Temperature, pH, and nutrient supplementation were all optimised for biodegradation determined from previous laboratory experiments. These remained constant within and between amendments.

Table 25. Hydrocarbon content of Derwenthaugh soil sampled at different depths.

Hydrocarbon content of Derwenthaugh soil (30g) was determined by extraction with CCl_4 for 12h followed by i.r. analysis of the diluted extracts.

Soil Depth (cm)	Extractable hydrocarbon (mg/g)*
<u>Derwenthaugh</u>	
surface	137.0±3.73
15	58.5±1.53
21	46.5±1.42
30	67.5±1.61
<u>Uncontaminated soil</u> (from adjacent field)	
surface	0.57±0.2

*mean of three replicates

4.5.10 Addition of laboratory-grown biomass or arable soil to DWH soil.

In the unamended DWH soil, both at its original pH value of 5.5 and after adjustment to pH 7.0, there was little, if any disappearance of phenolic material (4.0±0.6% and 9±4.5%, respectively) over a 6 week period. DWH soil was mixed with arable soil (50% by weight, pH 7.0) which resulted in the initial measured phenol concentration being reduced by 50%. The phenols extracted from arable soil were negligible in comparison to the contaminated soil and varied by less than 0.5% over the 6 week experimental period. The DWH-arable soil mix showed a decrease in residual phenolic concentration of 26±6.1% in the same period (Fig. 44). The addition of bacterial biomass, previously grown with phenolics, to 10^6 organisms/g soil led to a marked stimulation of phenolic degradation in the soil (26±5% loss in 2 weeks). The degradation rate then slowed but a second application of biomass at 21 days again accelerated phenolic loss until at 6 weeks, 47±7% of the original content had disappeared. The final (mean) arithmetic rates (ie over 6 weeks) were statistically different ($p < 0.05$). Cresol itself (1mM) when subsequently added to the biomass-ammended soil at 6 weeks disappeared within 7 days, indicating the continued activity of the introduced organisms, 3 weeks after addition.

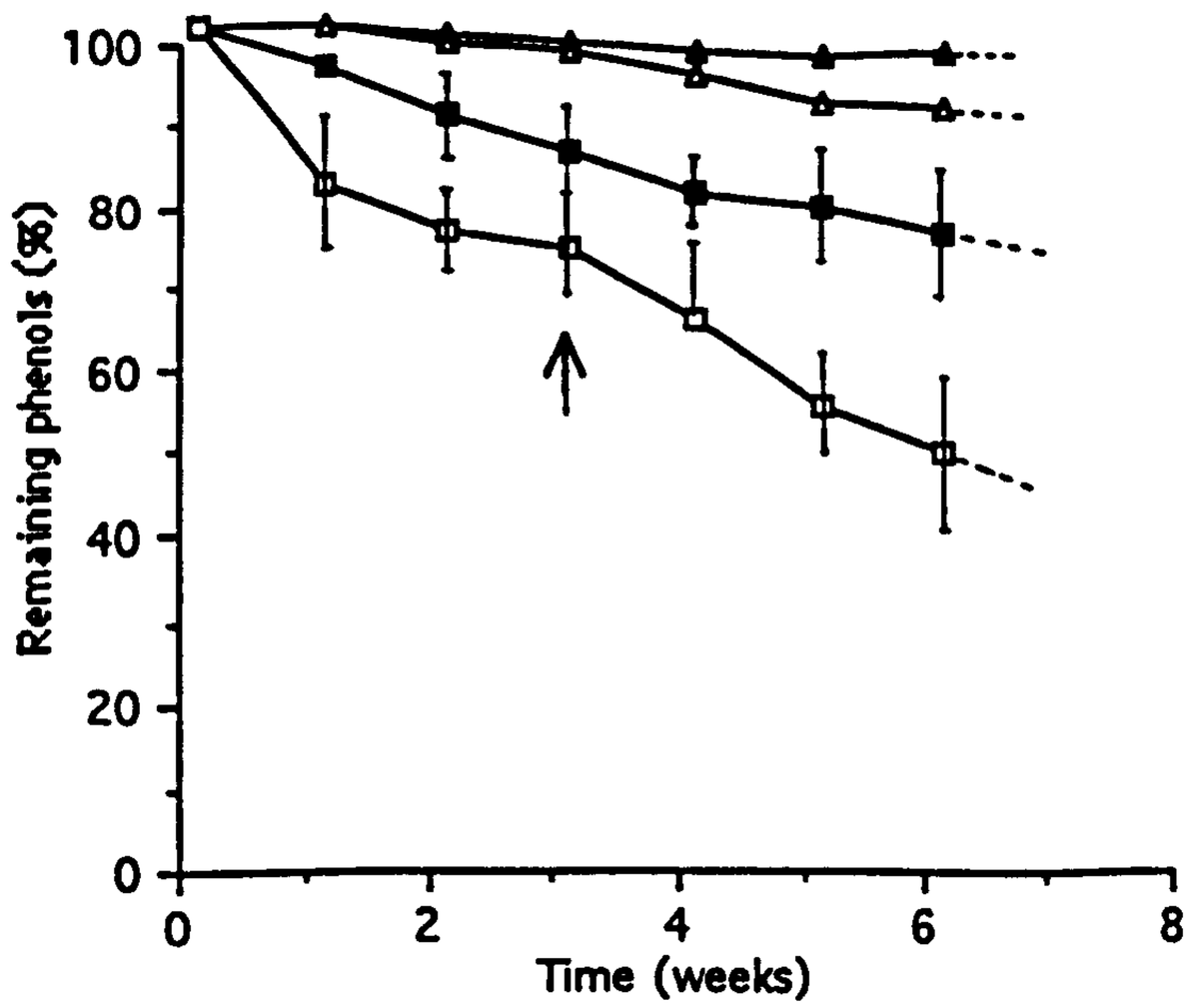


Figure 44. Loss of phenolic compounds from Derwenthaugh soil.

Pyrex dishes containing either, DWH soil pH 5 (▲); DWH soil pH 7 + minerals (Δ); DWH soil:arable soil (50:50) pH 7 (■) or DWH soil pH7 with nutrients and biomass added (❑) (arrow indicates the further addition of biomass to this jar), were stacked on stainless steel supports inside glass jars with screw-top lids (Section 2.6.12). Soils were stirred by hand every 2-3 days and experimental dishes were returned to their original fresh weight with the addition of distilled water kept at incubation temperature. Dishes were rotated regularly in the jars. Dishes were removed weekly and total phenolic compounds monitored by alkaline extraction followed by organic extraction and HPLC (Section 2.6.13). Error bars indicate standard deviations above 5%.

DISCUSSION

The environmentally acceptable remediation/disposal of contaminated land and contaminated sediments (from canals, rivers and lakes) is a world-wide problem.

A number of approaches to the treatment of contaminated sediments have been published, from low technology operations adopted by Turkey, for example, where contaminated sediment is dumped as hazardous waste (Alyanak, 1990) to high technology solutions such as that adopted by The Netherlands where separation of non-polluted sand from contaminated grains is effected by centrifugation (Van Luin & Stortelder, 1990) (Introduction p 27).

In the UK, in addition to the requirements of BATNEEC (best available techniques not entailing excessive cost), the DOE have emphasized the importance of end-use in determining the method and degree of site remediation. In the case of the Tyne dredgings, the site was to be partly landscaped and partly used for subsequent domestic housing/gardens, while the Derwenthaugh site was to be redeveloped into parkland. Where subsequent cultivation was intended environmental factors restricting plant growth on the contaminated sediment or soil would have to be overcome.

One of the many regions in the UK where sedimentary contamination presented a problem was the River Tyne. Tyneside has many areas of contaminated land as a result of the long history of heavy industrial activity in the region. Much of this industrial activity was known to have produced heavy metal and organic pollution, through direct discharge into the river and run-off from industrial sites, e.g. coking gas sites, which gave rise to significant organic pollution. These were originally thought to be the source of the pollutants in the River Tyne dredgings. The dredgings, on analysis showed a silty clay loam (70% silt, 23% clay), deficient in N and P. This, together with a lack of soil structure and the phytotoxicity of the metals present, were the main obstacles to the successful establishment and maintenance of vegetation on the

dredgings. Organic contamination, in particular phenol, found by previous workers (Pattinson, 1984; Johnson, 1987) was evident in the dredgings, but at concentrations below the DOE's threshold levels (Table 9), possibly due to degradation, leaching or volatilisation, that may have occurred during the intervening period.

Uncontaminated soils normally contain background levels of metals. Zinc, cadmium, copper and lead values for the control (arable) soil were at the lower end of this spectrum (Table 13). Levels of lead and copper in the River Tyne dredgings, in contrast, were above such background levels and bordered on the DOE's (1987) threshold levels. Elevated levels of zinc and cadmium were the predominant contamination in the dredgings and were considerably greater than DOE's (1987) threshold levels for domestic gardens and significantly above background concentrations found in uncontaminated arable soils at 30-fold and 13-fold respectively (Table 10).

The levels of heavy metal pollutants found in the dredgings were in broad agreement with earlier analyses carried out by Pattinson (1984) and Rimmer (1985), who both found high total and extractable levels of cadmium and zinc. As the level of heavy metal contamination was relatively high it could pose human health problems if allotments or vegetable gardens were established on sites covered with the Tyne dredgings. Alloway, consultant to Rimmer (1985a), recommended the incorporation of organic matter and lime to decrease the availability of metals to plants in the Tyne dredgings. Alternatively he suggested dilution of the dredgings with clean soil or covering the dredgings with uncontaminated top soil.

The approach adopted in this thesis to examine remediation methods for the River Tyne dredgings has been to employ a number of low cost amendments. These included addition of fertiliser, organic matter, lime (to modify pH) and combination of these three; weathering; and dilution with clean soil. The significance of heavy metal concentrations in the dredgings depended largely on how much of the various metals

were available for uptake by crops. Plant available metal in soil was assessed directly by means of plant analysis and indirectly by means of soil analysis, plant growth rate and yield.

Plants grown on the dredgings exhibited different tolerances to the metals present. Yields of rye-grass and lettuce were not significantly different to those grown on arable soil, although growth rates of lettuce grown on the dredgings were depressed (Table 11, Fig. 21). This is in agreement with the work of Bolton (1975) and Chadwick (1987). Low levels of phenol that were added to soil compost to mimic phenol levels occurring in the freshly-obtained dredgings (see p109) had a stimulatory, rather than inhibitory, effect on the growth of ryegrass (Table 11). Usually the presence of a pollutant has a negative effect on both microorganisms and plants, but low concentrations of compounds usually regarded as pollutants, may at times exert a stimulatory effect on microorganisms which can use them as nutrient sources. Phenol is produced by many plants in early stages of growth and can act as a stimulant to some plants (Vaughan *et al*, 1983; Whitehead *et al*, 1981). This effect may thus be due to enhancement of the bacterial population in soil, particularly in the rhizosphere.

Barley was chosen as a bioassay tool because of its sensitivity to the heavy metals present in the dredgings, although it was thought unlikely to be grown on the reclaimed dredgings. Unlike rye-grass or lettuce, appearance and yield of barley grown on the dredgings was markedly different from barley grown on uncontaminated soil.

Barley, grown on the Tyne dredgings, exhibited classic signs of metal phytoxicity with growth rates and yields severely depressed (Figs. 22, 23). The average barley growth rate and yield were 35% and 58%, respectively, less than the control ($p<0.05$). In plots with added spent mushroom compost there was a marginal improvement in growth rate over those in the dredgings alone, although overall yield of barley was not effected. Straw proved ineffectual as an ameliorating amendment until it had been further degraded.

Significant concentrations of zinc, cadmium and copper were found in the leaves of barley grown on the contaminated dredgings (Figs. 26, 27). In comparison, metal levels found in barley grown on control (arable) soil were lower than the usual range found in plant tissue (Table 14). The metal levels found in the plants grown in the dredgings were consistent with the observed phytotoxic effect at pH 6.7. The ready absorption of zinc, cadmium, copper and lead by barley under different soil conditions (Figs. 26, 27) was in agreement with the results of Smilde *et al.*, (1982) and is an indicator of the likely transfer of heavy metals into the food chain.

Raising the pH of the dredgings to between 7.1 and 7.3 by the addition of lime increased growth rates, yield and root length of barley grown in all the limed plots of the dredgings (Figs. 22, 23). Average barley yield increased by 47% over unlimed dredgings ($p<0.05$). In limed plots with spent mushroom compost and straw, growth rates and yield were not significantly improved over those of either the weathered fertilised dredgings or arable control soil. The mean amount of zinc, cadmium, copper and lead extracted by EDTA was reduced by 11.0%, 11.5%, 7.4% and 7.4% respectively in the limed dredgings compared with unlimed dredgings (Figs. 24, 25). This concurs with the findings of Sanders & Kherbawy, (1987) who reported that concentrations of zinc extracted from soil decreased as pH increased above a threshold value ranging from pH 6.2 to 7.0.

Mean zinc and copper levels in barley leaves grown on the limed dredgings in 1990 were lower than their previous 1988 levels and levels in barley grown in the unlimed soil by 23% and 16.6% respectively (Figs. 26, 27). It was apparent that these reductions of metals levels in the barley were sufficient to reduce metal levels in the plants to below critical levels. These values are in agreement with Davis *et al.*, (1978), who found critical levels for zinc of 160-320μg/g and for copper of 18-21(μg/g) in barley.

The addition of organic matter, (with a subsequent increase in cation exchange capacity) to soil, can have a mediating effect on the toxicity of metals to plants by providing natural organic chelating agents which increase the amount of available binding sites for heavy metals and thus reducing their uptake by plants. Extractable zinc, cadmium, copper and lead levels in plots of dredgings with organic amendments were similar to or slightly lower than levels in the dredgings alone in both 1988 and 1990 (Figs. 24, 25). This was reflected in lower levels of zinc and copper in barley leaves in 1988 and 1990 (Figs. 26, 27). There was, however, very little difference between levels of lead or cadmium. The mediating effect of soil organic matter in reducing the availability of metals in the plants is supported by the work of Halstead *et al.*, (1969); Alloway & Morgan, (1986); Piccolo, (1989); Guidi *et al.*, (1990) and Andersson & Siman, (1991). However, in the case of my experimental results, the reduction in metal levels in both in soil and plant may have been partly attributed to dilution effects, particularly as the growth rate and yield of barley in plots with organic amendments were not significantly different from growth rate and yield of barley grown in plots of dredgings alone.

Raising the pH of the dredgings by liming was one of the most effective means of minimising adsorption of heavy metals by barley. Albasel & Cottenie (1985) raised the pH of soils contaminated with zinc, copper and lead by liming; this appeared to be more efficient than peat or chelating agents (EDTA and DTPA) for reducing plant absorption of those metals. In soil, the single most important factor affecting the toxicity of metal pollutants to plants is thought to be pH and its consequent influence on the mobility and bioavailability of heavy metals (Page, 1981). pH plays a central role in determining the ionic status of the metal which influences the solubility and mobility of metals. Low pH increased the solubility of cadmium, zinc, lead, and copper by determining the chemical speciation of the metal, the different forms of which may have different toxicities (Bååth, 1989).

The extent of complexation of metals with organic and inorganic soil components also influences their toxicity because these derivatives are less toxic than free ions. Raising the pH increases the stability of most metal-organic complexes and increases ionization of the functional groups. At a high pH, carboxyls and phenolics react as acids and thus retain metallic cations (Cottenie, 1981). In the same way, liming reduces the availability of metallic cations and increases the availability of the anionic trace elements.

In conclusion, although the soil system is complex it is generally recognised that a decrease in soil pH will increase the solubility of cadmium, zinc and copper which in turn increases crop uptake of the elements affecting growth rates and yield. This was very evident from my own results, (Figs. 22, 23; 24, 25 and 26, 27).

Mixing with clean soil is one of the cheapest and easiest ways to reduce the levels of contamination of a polluted soil to below phytotoxic levels. Mixing the dredged material with uncontaminated soil compost decreased the overall concentration of available metals present so that the concentration of metals in the mixed soil decreased in proportion to the amount of soil compost added. The metal-rich dredgings needed to be diluted by 50% with fresh soil compost to achieve an equivalent yield to control soil (Fig. 28). Substitution of dredgings for soil compost raised heavy metal concentration in the plants grown on the mixtures, but there was a pronounced difference among elements. Plant zinc, and to a lesser extent copper and cadmium, were positively related to substrate content, the levels decreasing with decreasing proportions of dredgings in the soil. There was little relationship, however, between lead levels in soil and those in plants (Table 16). This is in agreement with the findings of other workers who found limited translocation of Cd and Pb to aerial shoots (John & Van Laerhoven, 1972; Påhlsson, 1989) and accumulation of Cd, Cu and Pb in the roots (Hardiman *et al,* 1984) due to possible binding of metals to root surfaces and cell walls.

In the Derwenthaugh soil, pollution appeared to be the result of the deposition of organic waste from coke production, as only organic pollutants were present above general background levels. Contamination appeared to be the result of surface spillage (Table 25). Phenols and polyaromatic hydrocarbons were in abundance and well above the DOE`s (1987) threshold levels (Table 17). Phenols cause necrosis, damage membranes and act as general cell poisons, while polyaromatic hydrocarbons may be carcinogenic so even relatively small amounts are a public health concern if present on land which is to be redeveloped for parks or domestic use. As a tributary of the River Tyne ran through the site adjacent to areas of contamination, particular attention was paid to water-soluble contaminants, especially toxic phenolics, for which the acceptable levels in drinking water are very low (1μg/l).

The approach adopted in this study to the problem of the remediation of organic soil contamination was to attempt to enrich and isolate bacteria from the Derwenthaugh soil capable of degrading the organic contaminants; specifically, phenols in the presence of thiocyanate were examined, as both of these occur in significant amounts in coking plant effluents. The enriched mixed bacterial culture comprised three *Pseudomonas* species (plus one other tentatively identified as a *Flavobacterium* species) (Table 22, Figs. 39, 40) which could degrade a range of phenols (Fig. 42), as well as those found in the Derwenthaugh soil.

The chemical structure of phenolic organic pollutants is a major factor in determining their persistence. Both the degree of substitution and the position of the substitutents on the aromatic ring of phenols were major factors in determining their ready biodegradability by the bacterial mixed culture enriched from the coke-work contaminated soil. The cresols were oxidised most easily, followed by dimethyl- then trimethyl-phenols (Figs. 42, 43). The position of the methyl substituent on the aromatic nucleus was also important in determining rates of substrate removal. In general, phenols with methyl groups substituted at positions 2 and 4, or 4 alone, underwent

degradation more rapidly than substituents in other positions, particularly 3 and 5. Higher oxygen uptake rates were observed with *ortho*-cresol and *para*-substituted methylphenols (*p*-cresol, 2,4-dimethylphenol) than with phenols having methyl groups in other positions (Table 24). Alexander, (1965) also indicated the increased recalcitrance of *meta*-substituted compounds to biodegradation. Tabak *et al.*, (1964) Goulding *et al.*, (1988); and Liu & Pacepavicius (1990), all report very similar findings, indicating the possibility of a relationship between chemical structure and oxidation rate.

Microorganisms play a key role in biogeochemical processes in the soil (i.e., the degradation, recycling and biosynthesis of biochemicals). Any factor unfavourable to the growth and proliferation of microorganisms will affect the biodegradation, and thus the persistence, of the pollutant concentration in the soil (Babich & Stotzky, 1977a; Hutzinger & Veerkamp, 1981). Degradation of organic pollutants in soil is usually dependent on microorganisms. Numbers of bacteria in the contaminated soil of the Derwenthaugh coking plant were significantly lower (10^6 bacteria/g soil) with associated poorer species diversity than those found in arable (control) soil (10^9 bacteria/g soil) (Table 21). This is in agreement with the findings of Doelman & Håånstra, (1979b) who in addition to lower numbers found lower metabolic activity in contaminated soil compared with fertile soil.

The insolubility and hydrophobicity of many aromatic compounds present a challenge to microorganisms regarding substrate uptake. It was notable that only a small percentage (6.1%) of infra-red recorded hydrocarbons in the DWH soil were degraded by sewage bacteria in 21 days compared with 16.2% degraded by bacteria enriched from the DWH soil (Table 20). Microorganisms attacking hydrocarbons frequently produce surface-active agents that emulsify hydrophobic compounds thereby increasing surface area and the bioavailability of substrate (Watkinson, 1980; Leisinger, 1983). There is evidence that microorganisms produce solubilising agents that appear to

be molecule-specific and allow hydrocarbons to pass through the cell wall and plasmalemma (Smith 1991). The use of surfactants to enhance biodegradability has been suggested (Bewley *et al.*, 1989) though the effectiveness and long term behaviour of such compounds has yet to be established.

Successful bioremediation either encourages native microbial flora to degrade the pollutant more rapidly or requires inoculation of the environment with novel microorganisms known to metabolise the chemicals readily. Enhancement of the growth of naturally-occurring organisms in the Derwenthaugh coke work-contaminated soil by improving environmental conditions, (such as, temperature, aeration, moisture level and mineral addition), accelerated biological degradation of organic contaminants (Fig. 44), particularly when the DWH contaminated soil was diluted with clean soil. This is consistent with findings from Crowder, (1991) and Lund & Gudehus (1990). Factors such as content of organic matter and clay, moisture level, temperature, pH, aeration and nutrient status have been found to be of importance in encouraging successful remediation (Torstensson, 1988). An adequate supply of oxygen is a further essential requirement for the metabolism of aromatic compounds and aliphatic and carbocyclic hydrocarbons by microorganisms, as they require oxygenases to initiate molecular attack and ideally completely oxidise the pollutants to CO_2 and H_2O.

Addition of microbes would be expected to aid biodegradation because of the limited number of specialist strains in a polluted and unimproved soil, able to exploit the pollutant(s). Inoculation of DWH coke work contaminated-soil with bacteria enriched on cresol and thiocyanate under laboratory conditions stimulated biodegradation rates of the contaminating phenols, resulting in decreased levels of phenolic compounds (Fig. 44), though repeated inoculations were necessary. Adding a readily degradable carbon source (yeast extract) along with inorganic nutrients proved to be beneficial and consistent with the work by Lindstrom & Brown, (1989) and Ying *et al.*, (1990) who both reported enhanced biodegradation of pollutant by addition of

an additional carbon source. Under the nutrient-limitation conditions usually operative in soils, there is a substantially weaker operation of catabolite repression and it may be possible, under such circumstances, for an organism to utilise two carbon substrates simultaneously (Painter & King, 1985).

Both successes and failures have been reported when species capable of destroying organic compounds in culture are added to samples in the natural environment (Goldstein *et al.*, 1985). The successful use of microbial inocula to enhance the degradation of aromatic contaminated soil has been reported (Morgan & Watkinson, 1989; 1989a). Various companies market microbial inoculant materials that are believed to increase hydrocarbon degradation in soils (Johnson *et al.*, 1985; Bartha, 1986). Molnna & Grubbs (1989) claim successes with several commercial products. When Vecchioli *et al.*, (1990) inoculated hydrocarbon-degrading bacteria into soil, these supplements were able to compete with the soil microflora and survive for the 60-day experiment, resulting in increased hydrocarbon biodegradation (65% hydrocarbon loss; 22% over that of uninoculated soil). Venosa *et al.*, (1991) used microbial inocula for cleaning up the Exxon Valdez spill which also offered some improvement over the rates catalysed by the indigenous organisms. In other studies, repeated inoculations were needed to maintain biodegradation rates (Jobson *et al.*, 1974; Staps, 1989a; Ellis *et al.*, 1990). It is pertinent that in the bacterial amendment experiment (Fig. 44) that the addition of phenol-degrading bacteria specifically enhanced removal of phenols but had only a small effect on hydrocarbon levels.

Attempts to accelerate the biodegradation of hydrocarbons resulting from oil spills by the introduction of commercially-available bacterial inocula have not, in general, enhanced the degradation rate above that which could be brought about by indigenous microorganisms (Prince, 1991), suggesting that the prevailing environmental conditions rather than the inoculum were the limiting factors (Finn, 1983; Atlas & Bartha, 1981). Jones & Greenfield (1991) added nutrients and bacteria

to soil contaminated with fuel oil. Addition of nutrients alone markedly stimulated biodegradation but the bacteria alone did not. As introduced microorganisms may face competition, predation or parasitism, they may be unable to compete efficiently with indigenous microflora for existing nutrients and are consequently unable to survive unless nutrient supplements are also added. Degradative capacity in soils is sometimes lost or repressed, however, if they preferentially utilise more readily degradable carbon sources.

In the case of xenobiotic compounds, particularly if recently released into the environment where indigenous microflora have not had sufficient time to adapt, introduced microorganisms capable of the successful metabolism of the xenobiotic(s) should be able to exploit the newly-created ecological niche successfully (Focht, 1987). While this has sometimes been achieved by deliberate genetic manipulation in genetically-engineered microorganisms (GEMs), it probably occurs naturally in the environment (Don & Pemberton, 1981; Slater & Lovatt, 1984; Van Elsas *et al.*,1987; Bale *et al.*, 1988) especially now that the genetic techniques, including the construction of gene cassettes made up of genes coding for enzymes with a broad substrate specificity, have been constructed to produce more efficient degradative strains for tackling recalcitrant compounds (Trevors *et al.*, 1987). Their use has led to concern about the survival of GEMs in the environment and dissemination of their genetically-engineered DNA sequences. Recent studies suggest that introduced GEMs can survive and transfer genetic information in soil and water environments (Stotzky *et al.*, 1989; Henschke & Schmidt, 1990; Chaudry & Chapalamadugi, 1991). The survival and persistence of introduced genetically-engineered organisms largely depends on the interaction with the indigenous microorganisms. The transfer of genes to an indigenous host would increase the chances of survival of the host bacteria, as bacteria that are found naturally in terrestrial habitats are highly likely to survive, whether or not the DNA they contain has been constructed by recombinant technology (Fry & Day, 1990).

Beringer & Bale (1988) have shown that competition for nutrients, parasitism and predation all influence survival of the organism. Soil type, nutrient levels, temperature and moisture levels are particularly important factors in favouring plasmid transfer in the soil (Klingmuller *et al.*, 1990; Top *et al.*, 1990; Lorenz & Wackernagel, 1991). Because of these factors, multiplasmid organisms in the absence of selection processes rarely compete well with wildtype or indigenous strains.

In conclusion, the contamination of the two samples of material examined have been shown to be the result of heavy metals in the river dredgings and organic hydrocarbons and phenolics in the DWH soil, and needed distinct approaches to remediation.

Zinc, cadmium, copper and lead readily accumulated in barley grown on metal-rich dredgings. Lime application to the metal-rich dredgings to raise the pH from 6.7 to 7.2 reduced the plant availability of zinc, cadmium, copper and lead; reduced the accumulation of zinc and copper, and prevented metal toxicity symptoms in the plants. Plants in limed soil showed a significantly beneficial effect in terms of growth rate and yield. Organic matter added to metal-rich dredgings did not significantly reduce plant availability or accumulation of metals in plants. Phenols added to these dredgings had no synergistically deleterious effect.

Crops grown on mixtures of dredgings and soil compost accumulated in the foliage Cu, Zn and to some extent Cd, according to the increase in the proportion of the dredgings component of the soil mixture. There was little congruency between plant and soil Pb.

Soil containing appreciable amounts of organic contamination in the form of phenolics was partially remediated by improving environmental conditions favourable to their degradation. Evidence has also been presented (in this thesis) to support the hypothesis that indigenous bacteria, separately cultivated and added back to the soil, can increase the rate of degradation of phenols.

While complete in itself the ideas and techniques used in this study offer wide scope for future work. Of the various pathways of exposure associated with contaminated land the soil pathways are among the most important but least understood. Soils serve as a sink for many organic contaminants because organic contaminants, particularly those with low water solubility, tend to sorb onto organic matter and clay particles in the soil.

Although the identification of organic pollutants was undertaken, their degradation, both chemical and biological, the extent to which their effects increase or decrease with time and their effect on plant growth and development would be pertinent areas of investigation and need to be established. The field application and monitoring of cultured bacteria enriched on such organic contaminants would be an area where the study could be carried forward. While the re-establishment of plant and animal species both by introduction and natural invasion could be monitored providing an insight into biodegradation and metabolism of contaminants by higher organisms (eg. assays of toxins in earthworms).

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Appendix 1. Results from regression analysis of the semi-log plots of biodegradation experiments as described in Sections 4.4.3 and 4.5.7.

i) Figure 34

2,4,6 trimethylphenol concentration (mM)	Slope	Linear regression co-efficient
0	- 0.002	- 0.928
0.5	- 0.142	- 0.988
1.0	- 0.143	- 0.959
2.0	- 0.055	- 0.968

ii) Figure 35

3,4,5 trimethylphenol concentration (mM)	Slope	Linear regression co-efficient
0	- 0.000	- 0.392
0.5	- 0.144	- 0.948
1.0	- 0.055	- 0.904
2.0	- 0.010	- 0.906

iii) Figure 42

Phenol	Slope	Linear regression co-efficient
cresol	- 0.083	-1.000
3,4 dimethylphenol	- 0.042	-0.893
3,5 dimethylphenol	- 0.026	-0.759
2,6 dimethylphenol	- 0.018	-0.906
2,3 dimethylphenol	- 0.018	-0.906
2,5 dimethylphenol	- 0.006	-0.760
2,4,6 trimethylphenol	- 0.001	-0.957
3,4,5 trimethylphenol	- 0.000	-0.571

iv) Figure 43

Phenol	Slope	Linear regression co-efficient
control	-0.000	- 0.958
p cresol	-0.042	- 1.000
3,4 dimethylphenol	- 0.004	- 0.975
2,5 dimethylphenol	- 0.001	- 0.953
3,4,5 trimethylphenol	- 0.0001	- 0.804
2,4,6 trimethylphenol	- 0.006	- 0.976